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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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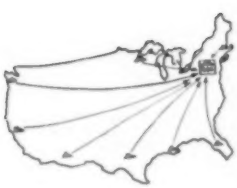

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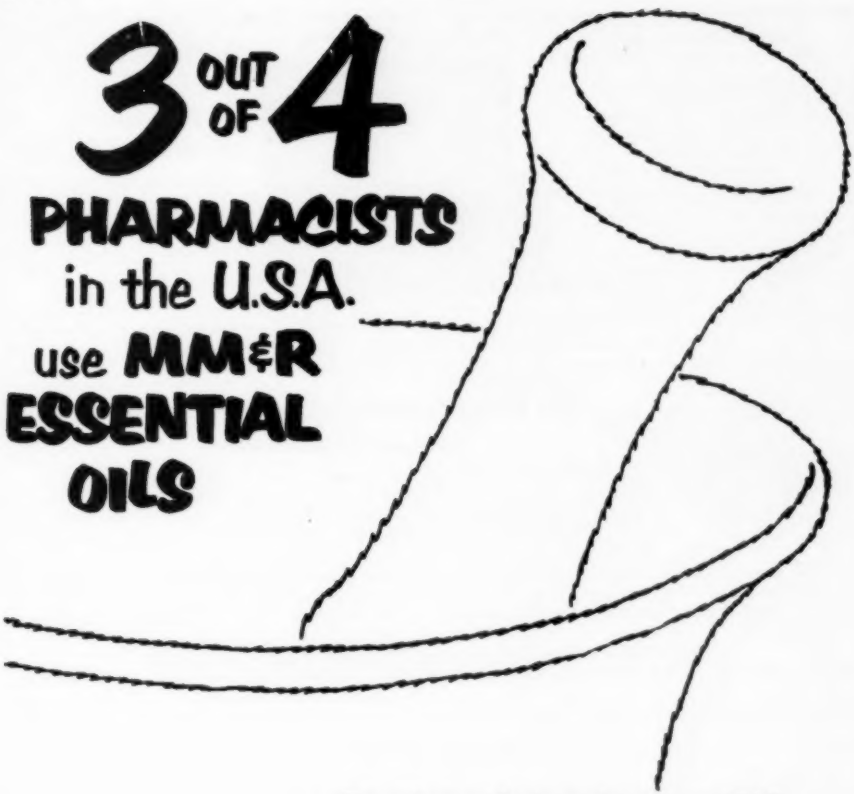

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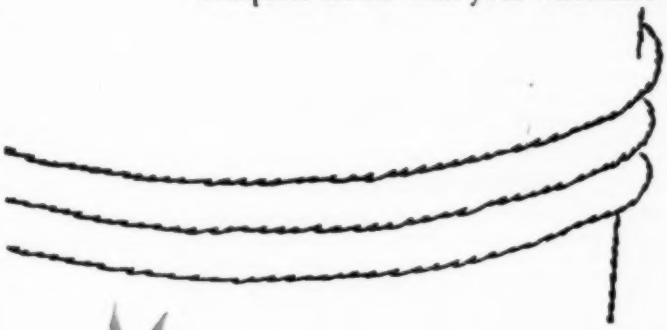

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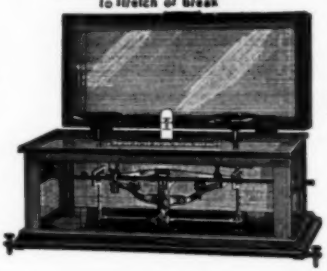

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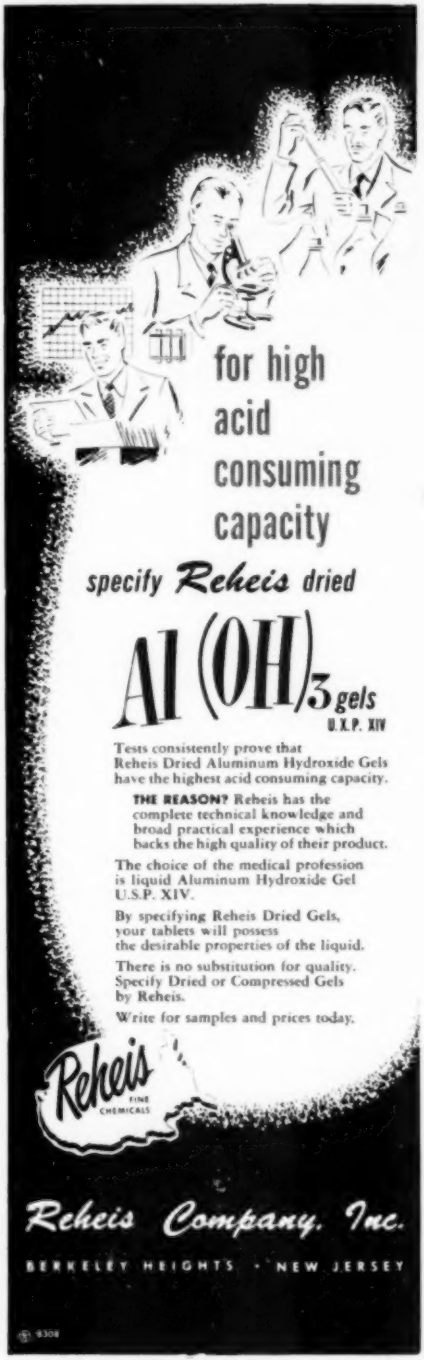
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VOLUME XLI — *APRIL, 1952* — NUMBER 4 — CONSECUTIVE NO. 8

Separation and Analysis of APC Combination by Partition Chromatography*,†

By T. HIGUCHI and K. P. PATEL

A method of separating and analyzing mixtures of phenacetin and caffeine suitable for the analysis of APC-type combinations is presented. The separation is obtained by means of a simplified partition chromatographic column with water as the internal phase. The analysis of the eluate is achieved by a spectrophotometric method. Results found for known simple mixtures are superior to those obtained with previous methods.

THERE is at present no simple and rapid method for determining the constituents of mixtures of aspirin, caffeine, and phenacetin. A rather successful attempt has been made recently in analyzing these mixtures by infrared spectrophotometry (1); the method, however, requires equipment not ordinarily found in an average laboratory and is, furthermore, rather time-consuming. The primary difficulty encountered by other workers in this field has been in separating caffeine from phenacetin. A number of extractive methods have been proposed (2), but they are generally either too time-consuming or inadequate.

An attempt was made in the present investigation to develop a simple chromatographic method of separating caffeine from phenacetin. This separative technique was chosen because of its successful application in our laboratories to a number of organic compounds which would have been otherwise exceedingly difficult to analyze.

SEPARATION OF CAFFEINE AND PHENACETIN BY PARTITION CHROMATOGRAPHY

In partition chromatography advantage is taken of the difference in the partition characteristics of the components for their separation. For the particular case at hand, a solvent combination of water and a nonpolar solvent can be used since caffeine has a definite affinity for water and considerably less affinity for nonpolar solvents such as ethers, whereas the contrary is true for phenacetin. In Fig. 1 is shown a partition chromatogram obtained from a 5-ml. sample containing 25 mg. of caffeine and 25 mg. of phenacetin by eluting with a mixture of diisopropyl ether and chloroform and then with chloroform. Water was used as the stationary phase. It is evident from the elution curve that it is possible to obtain a rather clean separation between the two components.

A number of solvent combinations were studied before the solvents (diisopropyl ether and chloroform) were chosen. These appear to give the best separation, possibly because one is a protophilic solvent whereas the other is a protogenic solvent, although both are essentially nonpolar in nature.

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† This project was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

ULTRAVIOLET ABSORPTION ANALYSIS OF ELUATE

There are several methods available for determining the solute contents of the eluted fractions. The data plotted in Fig. 1 were obtained by determining gravimetrically the solid content of each fraction. This method, although rapid and convenient, is suitable only if appreciable amounts of both phenacetin and caffeine are present. Another approach which proved quite feasible for milligram quantities of the constituents depended on spectrophotometric determination of the separated fractions.

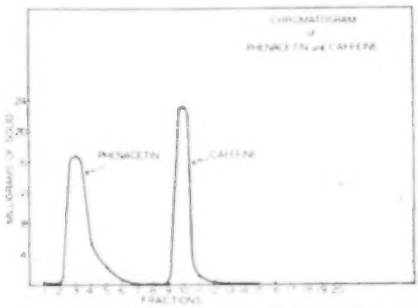

Fig. 1.—Chromatogram of a mixture of phenacetin and caffeine. Water was used as the internal phase; 75:25 (v/v) diisopropyl ether-chloroform mixture was used as the eluant for the first 70 ml.; and straight chloroform used as the eluant for the remainder. Fractions were 10 ml. in volume.

The ultraviolet absorption spectrum of caffeine as obtained on a Beckman Model DU Spectrophotometer is shown in Fig. 2, as well as that of phenacetin. It is evident from the spectra that ultraviolet spectrophotometry affords a means of detecting and determining exceedingly dilute solutions of both constituents. In the results reported in this paper, absorption readings at 275 mμ were used to determine the concentrations of caffeine, and those at 287.5 mμ for the concentrations of phenacetin. The absorption peak was not used in the latter case since it lies in that part of the spectrum less susceptible to quantitative measurements on the Beckman Spectrophotometer, the inflection point at 287.5 mμ being more suitable for this purpose.

The present method differs from previously proposed ultraviolet methods (3) in that it requires separation of the components before spectrophotometric measurements. This procedure is essential if accurate estimations of small amounts of caffeine are to be made in the presence of larger quantities of phenacetin.

EXPERIMENTAL

On the basis of the above and other studies, the following procedure was developed for separating and determining caffeine and phenacetin present in chloroformic solutions. Such solutions may be prepared either by extraction of weighed samples or by simple dissolution.

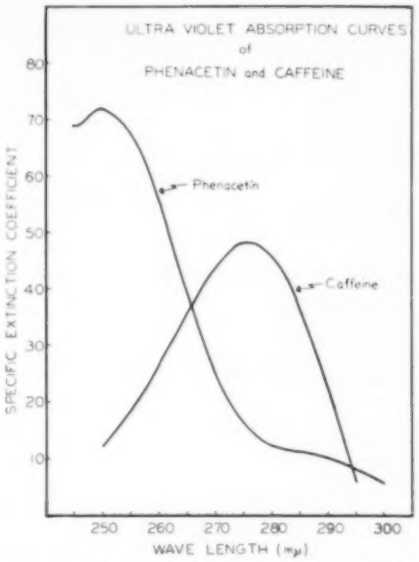

Fig. 2.—Ultraviolet absorption spectra of phenacetin and caffeine, as determined in chloroform with Beckman Model DU Spectrophotometer.

Apparatus.—A 20-mm. Pyrex chromatographic column 45 cm. long is used. A close-fitting glass plunger is employed in packing the column.

Packing of the Column.—20 Gm. of silicic acid (Mallinkrodt, chromatographic grade) and 20 ml. of the water are thoroughly worked together in a 400-ml. beaker with a spatula. Approximately 150 ml. of 75% diisopropyl ether (freshly distilled)–25% chloroform mixture is added and a homogeneous slurry formed by vigorous stirring. This is packed increment-wise into the column, care being taken to prevent formation of air pockets or other forms of heterogeneity. Each increment is packed down firmly with the plunger. Finally, a circle of filter paper is placed on the top of the packing.

Sample Addition and Elution.—The chloroformic solution of the sample is diluted three parts by volume with diisopropyl ether. Exactly 5 ml. of this diluted solution is chromatographed on the prepared partition column, the usual chromatographic precautions being observed in the sample addition and subsequent elution steps. The entire phenacetin contained in the added sample is obtained in a single fraction of 100 ml. by elution with the 75:25 diisopropyl ether-chloroform mixture. The caffeine component, which remains on the column, is then stripped out by elution with 100% chloroform.

Analysis of the Eluate.—The phenacetin content of the first fraction and the caffeine content of the second fraction are determined spectrophotometrically. A 10-ml. aliquot is taken from each and placed in a 100-ml. volumetric flask. The solvent is rapidly removed by swirling the solution under vacuum and the residue is redissolved with chloroform and diluted to the mark with the same solvent.

TABLE I.—RECOVERIES FROM KNOWN MIXTURES

Sample No.	Substance	Amounts of Each Substance Added to Each Column, Mg.	Recoveries, Mg. Column 1	Recoveries, Mg. Column 2
1	Phenacetin	13.50	13.60	13.50
	Caffeine	5.18	5.17	5.15
2	Phenacetin	44.32	42.88	44.42
	Caffeine	5.02	5.01	5.05
3	Phenacetin	42.31	41.78	41.78
	Caffeine	8.92	8.98	8.82
4	Phenacetin	39.99	40.24	40.09
	Caffeine	14.67	14.78	14.76
5	Phenacetin	28.32	28.17	28.27
	Caffeine	19.93	19.85	19.78
6	Phenacetin	20.26	20.12	20.31
	Caffeine	10.31	10.15	10.12
7	Phenacetin	27.19	27.07	26.63
	Caffeine	6.21	6.18	6.21
8	Phenacetin	23.96	23.85	23.75
	Caffeine	14.36	14.21	14.35

TABLE II.—DETERMINATION OF PHENACETIN AND CAFFEINE IN CERTAIN COMMERCIAL PRODUCTS

Sample	Substance	Amounts (Labeled) of Each Substance Added in Each Column, Mg.	Recoveries, Mg. Column 1	Recoveries, Mg. Column 2	Recoveries, Mg. Column 3
Eli Lilly capsules	Acetylsalicylic acid	45.4	...	...	...
	Acetophenetidin	32	32.6	32.2	31.8
	Caffeine	6.5	6.3	6.2	5.8
Eli Lilly tablets	Acetylsalicylic acid	45.4	...	...	...
	Acetophenetidin	32	34.7	33.6	33.1
	Caffeine	6.5	6.0	6.0	5.9
Burroughs Wellcome tablets	Acetylsalicylic acid	45.4	...	...	...
	Acetophenetidin	32	31.9	32.5	32.5
	Caffeine	6.5	5.7	5.8	5.8

The absorbancy of the resulting solution is determined at the chosen wave length on a Beckman Model DU Spectrophotometer. If the value obtained is greater than 0.9, the solution is diluted further with chloroform.

The exact phenacetin and caffeine contents of the original sample are then calculated from the absorbancy readings and the dilution factors based on the entire procedure.

In the given procedure only minimal care is necessary in cutting the two fractions since the phenacetin zone moves rapidly and is completely eluted before the first traces of caffeine appear in the eluate. The procedure requires very little of the operator's time since the column needs only minor attention during the elution process.

For the spectrophotometric determination of the components the procedure calls for removal of the solvents. This is necessary because some of the results obtained during the preliminary studies indicated that the diisopropyl ether used contained volatile impurities which interfered in the ultraviolet.

RESULTS AND DISCUSSION

In Table I are listed recoveries obtained by applying the given procedure to known mixtures of caffeine and phenacetin. Rather widely differing compositions were taken for this test purpose. The recovery data for both constituents appear to be quite satisfactory, the means of the duplicate runs showing less than 1 per cent absolute deviation on the average from the known values for both caffeine and phenacetin.

Where aspirin is present in the samples it is usually advisable to remove the acidic component first by extraction of the chloroformic solution with a mildly alkaline solution. The aspirin is then determined separately. If, however, only estimates of phenacetin and caffeine are desired, the samples can be chromatographed without removal of aspirin if a buffer solution, *p*H 6–7, is substituted for water as the stationary phase. The acidic component is retained firmly by the buffer phase, whereas the neutral components are essentially unaffected by this change. Results of some analyses of commercial preparations are given in Table II.

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The Effect of X-Irradiation on Intestinal Absorption and Thyroid Uptake of the Iodide Ion Using I-131*, †

By PATRICK F. BELCASTRO,‡ JOHN E. CHRISTIAN, and H. GEORGE DeKAY

Rats were exposed to whole body irradiation using sublethal doses of "hard" X-rays. After a three-day latent period, an aqueous solution of iodine-131 labeled sodium iodide was orally administered to the irradiated animals. The rate of intestinal absorption and thyroid uptake of the labeled iodide ion was studied by comparing the amount of radioactivity found in the intestinal contents and thyroid glands of irradiated animals with that of control or nonirradiated animals. Utilizing this technique, statistically analyzed results failed to reveal any appreciable change in the rates of intestinal absorption or thyroid uptake of the iodide ion.

RADIATION in some form has been used for therapeutic purposes since the beginning of mankind. The discovery of X-rays by Roentgen in 1895 and many developments of the 20th century have led to the present status of radiation research in the biological sciences. Some of the most important events include the discovery of artificial radioactivity by Joliet and Curie (1), the introduction of the cyclotron by Lawrence (2) and particularly the recent advances in nuclear physics. The increasing availability of atomic energy accompanied by an increasing number of personnel who are being exposed to ionizing radiations has focused a great deal of attention on this important subject.

This increased interest came into existence as a necessity for obtaining a better understanding of the actions of ionizing radiations on normal and pathological tissue, so as to better utilize their desirable properties as well as to counteract or prevent their undesirable effects.

Ionizing radiations have the property of electrifying or ionizing matter by removing electrons from atoms of elements which make up essential compounds utilized in the control of various body functions of the living system. The damaging effects of these radiations to living cells are accompanied by changes in function or cessation of activity. Since orally administered radioactive isotopes are employed as tracers of physiological processes in the intact animal, it was considered important to determine whether or not the ionizing radiations emitted from these substances were sufficient to cause any deviation in the normal rate of their absorption from the intestines. It was believed that this information could be derived from results obtained by exposing animals to acute doses of X-rays since all high energy radiations have the same kind of effect on living animals and tissues. This project was also undertaken to study the behavior of the thyroid gland of irradiated animals in regard to its ability to remove the iodide ion from the circulation and to establish and evaluate a technique by which the rate of intestinal absorption may be ascertained through the use of radioactive materials. Iodine-131 was chosen for this study because of its convenient half life (8.0 days) and because it is readily detected in the thyroid and other tissues.

Results of previous work reported in the literature revealed that whole body exposures to X-rays may have a series of pathological and hematological effects that can directly or indirectly stimulate activities of various organs, including the thyroid gland and the intestines. Results have generally been shown to be inconsistent, but to depend on the level of X-irradiation and on the nature of the material being studied. Evans, *et al.* (3), showed an increase in the thyroid uptake of subcutaneously administered radioactive iodine-131 after whole body X-irradiation in rats exposed to 500–1,000 Roentgens. Barron (4) investigated the effects of X-rays on the metabolism of the isolated small intestine and its permeability to glucose. He suggested the possibility that ionizing radiations may disturb the selective permeability of the intestinal cells. Beutel and Klein (5) mentioned that histamine-like bodies cause an increase in capillary permeability following Roentgen irradiation. Bloom (6) and Hagen (7) reported histopathological damage in the form of degeneration of the epithelium, loss of mitotic activity, and extreme damage to the crypts of the duodenum, after whole body exposure of rats to 600 Roentgens of X-rays.

The rate of intestinal absorption of iodine has been studied by numerous workers in connection

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‡ Fellow of the American Foundation for Pharmaceutical Education.

with oral administration of this material in iodine metabolism studies. Hamilton (8) studied the rate of intestinal absorption of some radioactive halogens by noting the change of radioactivity in human hands and concluded that complete absorption of iodine-131 occurs in two hours. Perlman, *et al.* (9), used radioactive iodine in absorption studies in connection with iodine metabolism and found that 80 per cent absorption occurred within three hours after oral administration.

Normal thyroid physiology and iodine uptake using radioactive iodine have been extensively studied by various workers. Marine (10), Hertz (11), and Leblond (12) demonstrated that the normal thyroid collects a greater proportion of administered labeled iodine than any other tissue. Hertz (13) and Hamilton (14) showed that the percentage uptake of radioactive iodine by the normal thyroid gland increases as the dose is decreased. Keating and Albert (15) stated that inorganic iodine disappears from the blood at an exponential rate which is the sum of the individual rates of removal by the kidneys, thyroids, and other tissues.

EXPERIMENTAL

White, male, adult, Purdue-Wistar strain rats were exposed to "hard" X-rays originating from a General Electric "Maximar" therapy unit[1] adjusted to deliver 52 Roentgens per minute under the following conditions: 220 kilovolts, 15 milliamperes, 50 centimeters focal film distance, and an area of irradiation of 30 x 30 centimeters. The filters employed consisted of a 0.5 mm. thickness of copper and 1.0-mm. thickness of aluminum. Four groups (40 animals per group) of animals each received single exposures of 130, 260, 520, and 624 Roentgens[2] of X-rays.

The animals were immobilized during the exposure by placing each animal in an individual nylon holder which was designed to permit only a slight movement of the head. These were secured to a wooden board 1 foot square and $^3/_4$ inch thick. Five animals were irradiated during one exposure.

After irradiation the animals were held in individual cages for a latent period of three days to allow the maximum effects of the irradiation to take place. After this time, a tracer dose (no stable carrier added) of sodium iodide, labeled with radioactive iodine-131, was orally administered. The administered radioactive aqueous solution had an activity of approximately 100,000 counts per minute[3] in a total volume of 0.5 cc.

After periods of one, two, three, and four hours the animals were sacrificed by chloroforming and the thyroids subsequently removed and macerated in 4 cc. of a 5% sodium hydroxide solution. The stomach and small intestines and their contents were removed and macerated in sufficient 5% sodium hydroxide solution to give a final volume of 50 cc. After thorough dispersion, the samples were allowed to stand twenty-four hours.

Aliquot portions of the clear liquids were transferred to tin foil counting cups having a diameter of 3.0 cm. Prior to counting, the samples were dried by means of an infrared lamp. All samples were counted within one or two hours, as was a standard dilution of the solution which was administered to the animals.

A suitable number of control or nonirradiated animals were treated in the same manner at the same time as were the irradiated animals.

The concentration of radioactivity found in the thyroid gland was taken as the total amount of radioactive iodide ion that was removed by this gland from the blood stream after each designated time period. The amount of radioactivity remaining in the intestinal and stomach tissues and their contents was taken as the quantity of unabsorbed radioactive iodide ion. All values were expressed as "per cent of the dose administered" and compared with those derived from control animals.

The symptoms observed within the post-irradiation period of three days were noted. The degree of body weight lost during the latent period served as the criteria for estimating the approximate extent of general physiological damage induced by each level of X-irradiation.

All results were statistically treated and the resulting data were analyzed for significance.

RESULTS

The effects of the irradiation treatment were manifested in the clinical symptoms that were exhibited within the three days following irradiation. The severity of the symptoms increased with an increase in the Roentgen dose. Anorexia, severe diarrhea, internal hemorrhage, and loss of weight were the most common symptoms observed. These and numerous other symptoms have been reported by Selle (16) and others and are characteristic of "radiation sickness."

A loss in body weight was observed in most of the animals. It was interesting to note that at the 624 Roentgen level, 100% of the animals lost weight. However, only 66% of the total animals irradiated lost weight at the 130 Roentgen level, indicating an animal variation in response to this lower dose. After 260 and 520 Roentgens of X-rays, approximately 90% of the animals lost some weight. Figure 1 shows the extent of body weight lost after exposure to increasing doses of X-rays.

In spite of the numerous physiological changes induced by the X-irradiation as manifested in the observed clinical symptoms, the rate of intestinal absorption of the iodide ion was not appreciably altered during the test periods. The concentration of iodide ion which penetrated into the blood stream from the small intestines of irradiated animals was not statistically greater than that of control or nonirradiated animals. The statistical treatment of the

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[1] The authors are indebted to Dr. Donald C. McClelland of the Arnett Clinic in Lafayette, Ind., for the use of his X-ray machine.

[2] Refers to dosage of X-rays measured in air.

[3] Radioactive determinations were all made by means of a Geiger-Mueller counter equipped with a thin mica window having a thickness of 2.7 mg./sq. cm. All radioactive samples were located 7.5 mm. distant from the thin mica window.

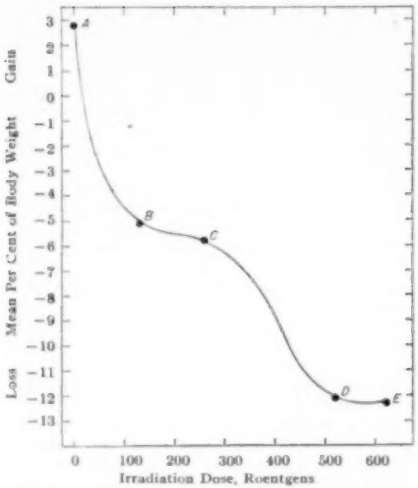

Fig. 1.—Effect of X-irradiation on body weight of rats. *A*, 16 animals; *B*, 26 animals; *C*, 34 animals; *D*, 20 animals; *E*, 44 animals.

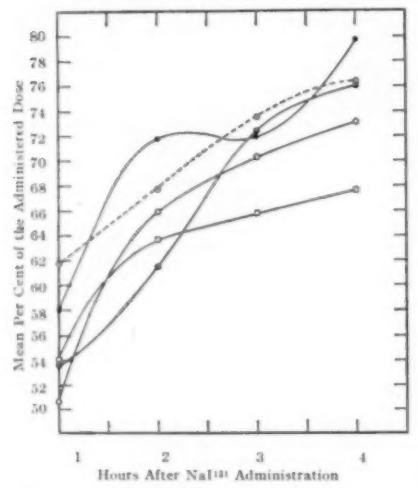

Fig. 2.—The rate of intestinal absorption of the iodide ion in control and X-irradiated animals.

⊙, control; ◒, 130 Roentgens; ●, 260 Roentgens; ○, 520 Roentgens; □, 624 Roentgens.

data included the computation of the Fisher and Yates "*t*" value (17) which compared the significance of difference between the mean per cent of labeled iodide ion absorbed in irradiated animals and the mean per cent absorbed in control animals. Figure 2 illustrates the close proximity in the rates of intestinal absorption of control and irradiated animals.

Similarly, the rate of thyroid uptake of labeled iodide was not greatly affected by the irradiation

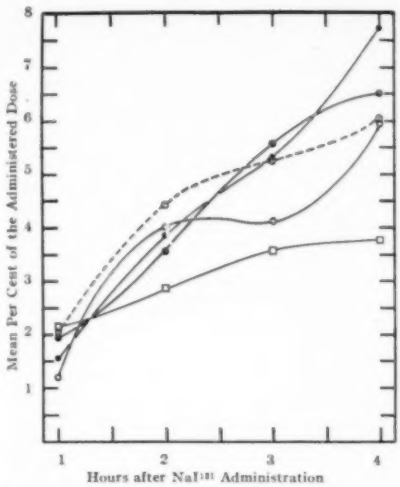

Fig. 3.—The rate of iodide ion uptake by the thyroids of control and X-irradiated animals.

⊙, control; ◒, 130 Roentgens; ●, 260 Roentgens; ○, 520 Roentgens; □, 624 Roentgens.

treatment. There was no apparent significant difference in the ability of the thyroid gland of control and irradiated animals to remove the iodide ion from the circulating blood. After exposure to 624 Roentgens, the highest dose to which the animals were exposed, there occurred a trend toward a decrease in the rate of iodide ion uptake by the thyroids as is shown in Fig. 3. However, these values only approached the statistically significant "*t*" value and therefore indicate that further study is necessary to support any conclusive statements regarding a decreased uptake of iodide ion by the thyroid.

CONCLUSIONS

1. Acute whole body X-irradiation in sublethal doses did not appreciably influence the rate of intestinal absorption of tracer quantities of labeled iodide ion when studied according to the technique described, using the rat as the test animal.

2. Similarly, the rate of thyroid uptake of radioactive iodine-131 as the iodide ion was not sufficiently altered by whole body X-irradiation to give statistically reliable data with which to support the difference between the mean per cent of the dose taken up by the thyroids of irradiated animals and the mean per cent of the dose of the control or nonirradiated animals.

3. With reference to conclusions 1 and 2, there is a need for studying the effects of ionizing radiations on isolated living tissues and organs. In the intact animal, whole body X-irradiation studies are made complex by the influence of

other integrated, compensating body functions and organs which affect the particular tissue being studied.

4. Intestinal absorption and thyroid uptake of the iodide ion in this work was studied three days after X-irradiation. The negative nature of the data does not suggest that they are applicable to possible similar studies carried out immediately or a few hours after X-irradiation.

5. Although the results of this work may be indicative of the effects of X-irradiation on the intestinal absorption rate and thyroid uptake rate of other inorganic halogen ions, further work is necessary to determine the effect of ionizing radiations on the absorption of other chemically different substances.

6. The rate of intestinal absorption can be efficiently studied by a suitably labeled radioactive material and subsequently determining the amount of unabsorbed radioactive material in the gastrointestinal tract.

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Application of the Karl Fischer Method in Medicinal Chemicals and Other Drug Products*

By EINAR BROCHMANN-HANSSEN and PHYLLIS PONG

The Karl Fischer reagent was used to determine the water content of various medicinal chemicals, powdered extracts, and ointments. In all instances, this method was found to be superior to the official methods for the determination of moisture. Both visual and electrometric end points were used. Chemicals and ointments could be analyzed satisfactorily by direct titration. Commercial powdered extracts were found to have a rather high moisture content, varying from 2.1 to 8.2 per cent.

SINCE ITS discovery in 1935, the Karl Fischer reagent has found extensive use in various industrial laboratories for the determination of water in a great variety of materials. Although this method became official in U. S. P. XIV, little work has been done as yet on the application and limitation of this technique to pharmaceutical products.

Biermacher and Mattocks (1) determined the moisture content of several medicinal chemicals official in the National Formulary by means of the Karl Fischer titration. In several instances the results differed considerably from those obtained by the usual oven drying method. Hulme and Osol (2) reported favorable results using the Karl Fischer technique for the determination of moisture in crude drugs. In the present work this method has been applied to a number of medicinal chemicals as well as to powdered extracts and ointments.

It is well known that most powdered extracts are very hygroscopic. By absorbing water their stability is often reduced appreciably, due to hydrolytic and enzymatic reactions. Powdered extracts with a high water content also give rise to many technical problems in prescription practice and pharmaceutical manufacturing. Many modern pharmacopeias, therefore, have established definite limits as to the moisture content of powdered extracts, these limit values ranging

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from 3 per cent in the Swiss pharmacopeia to 6 per cent in the Dutch pharmacopeia.

Zelger (3) compared various methods for determination of water in powdered extracts. He found that drying in a desiccator over sulfuric acid or phosphorus pentoxide gave much too low values. Oven drying caused difficulties because the extracts often contain small amounts of volatile materials other than water. Bouchardy and Mirimanoff (4) used infrared drying for powdered extracts and reported lower values by this method than by oven drying.

It may often be desirable to determine the water content of certain ointments and ointment bases both in order to detect adulteration and for reasons involving the stability or therapeutic value of the preparation.

The Food and Drug Administration (5) recommends the Karl Fischer method for determination of moisture in ointments containing antibiotics.

U. S. P. XIV determines the water content of wool fat and hydrous wool fat by drying to constant weight on a water bath. Molnar (6) used oven drying for ointments and emulsions, whereas Kedvessy and Träger (7) absorbed the water in anhydrous copper sulfate and calculated the water content from the increase in weight.

EXPERIMENTAL

When the Karl Fischer reagent is prepared in the usual way with methanol (8), it is very unstable and requires frequent standardization. This has detracted much from its general appeal. Work done at Shell Development Company, Emeryville, Calif. (9) has shown that the reagent is much more stable when prepared with ethylene glycol monomethyl ether instead of methanol. This was confirmed by us (Fig. 1).

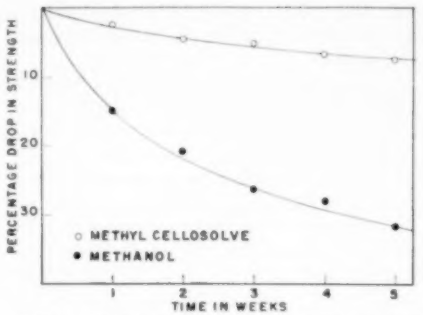

Fig. 1.—Deterioration of Fischer reagent prepared with methanol and with methyl Cellosolve as solvents.

Reagents.—The Karl Fischer reagent was prepared as described by Mitchell and Smith (8), but anhydrous ethylene glycol monomethyl ether[1] was used instead of methanol. One milliliter of this reagent corresponds to 3.5 to 4.0 mg. of water. The reagent was standardized against a known quantity of water weighed from a Lunge weighing pipette. When an indirect titration method was used, the excess of Fischer reagent was back-titrated with a solution of water in methanol prepared by diluting 3.5 to 4.0 Gm. of distilled water to 1,000 ml. with reagent methanol. This solution, which is stable when stored properly, was standardized against the Fischer reagent. It was also used to check the Fischer reagent from day to day. Reagent methanol (for ointments a mixture of methanol and toluene) was used as the solvent for the samples whose water content was determined.

Apparatus.—The titration assembly consisted of two Machlett Auto-burettes with 2-L. reservoirs and was protected against atmospheric moisture by large drying tubes filled with indicating Drierite.

For visual titration, the titration vessel was a 150-ml. Pyrex flat-bottomed extraction flask with a wide neck. It was fitted with a two-hole rubber stopper, one hole for the burette tip, the other for a drying tube.

The titration vessel for electrometric titration was similar to the one just mentioned, but had a standard taper joint 12/30 side arm through which a double platinum electrode was inserted. A magnetic stirrer was used, and a Beckman *p*H meter, Model H-2, with a polarizing adapter served to indicate the voltage change at the end point.

Procedures.—Visual titration was found to give reproducible results with soluble, colorless compounds when a color standard was used at the end point. The color standard was prepared by titrating 20 ml. of reagent methanol with Karl Fischer reagent to the desired end-point color in a 100-ml. Pyrex volumetric flask equipped with a glass stopper. A determination was carried out as follows: From 10 to 20 ml. of reagent methanol, depending upon the solubility of the substance to be analyzed, was pipetted into a dry titration flask and brought to the end-point color with the Fischer reagent. The flask was then removed from the burette tip, and the hole in the rubber stopper closed with a piece of glass rod to reduce the exposure to air. As rapidly as possible, the sample, usually containing from 25 to 75 mg. of water, was introduced and dissolved, and the solution was titrated to the end point again. On very humid days, and particularly with samples containing very little water, it was found necessary to run a blank in order to correct for the atmospheric moisture absorbed while introducing the sample.

For even higher accuracy or when the sample was colored but still soluble, a direct electrometric titration was used ("dead-stop" end point). The solvent was first brought to the end point, then the sample was introduced and dissolved, and the solution was titrated to the end point again. Ointments were conveniently dispensed from small collapsible ophthalmic ointment tubes.

This direct method was not satisfactory for powdered extracts because they were not completely soluble, and the water was extracted very slowly. However, good results were obtained by indirect

[1] Methyl Cellosolve, Carbide and Carbon Chemicals Corporation.

titration as follows: 20 ml. of reagent methanol was pipetted into the titration flask, a small excess of Karl Fischer reagent was added, and the mixture was stirred for one minute. The excess of Fischer reagent was then back-titrated with a standard solution of water in methanol. Approximately 0.5 Gm. of the powdered extract (accurately weighed) was introduced, followed by 20 ml. of Karl Fischer reagent. The mixture was stirred for a definite length of time, and the excess of reagent was back-titrated with water in methanol solution. A blank was run under the same conditions to correct for the moisture absorbed during the determination. Normally, this blank value was very small. For complete extraction of all water an extraction time of thirty minutes appeared to be necessary.

In order to determine the validity of the procedures just described, the compounds and preparations in question were assayed for water by an independent method. The official drying methods were used for chemicals, oven drying at 103–105° for extracts, and toluene distillation for ointments. For powdered extracts a combination of the Fischer method and the toluene distillation method was also used. The still is pictured in Fig. 2. Approximately 150 ml. of toluene was introduced into a 200-ml. round-bottomed flask equipped with a standard taper 24/40 opening. The flask was connected to the condenser, and approximately 50 ml. of toluene was distilled over in order to remove traces of moisture from the toluene and from the still itself. After cooling for a few minutes, the sample of powdered extract, containing not more than 15–20 mg. of water, was introduced. From 50 to 75 ml. of toluene was now distilled into the titration flask containing 10 ml. of methanol which had been brought to the end point with Karl Fischer reagent (back-titration). The water that had distilled over was titrated by the back-titration method because the large volume prevented a sharp end point by the direct method.

The results are summarized in Tables I, II, and III.

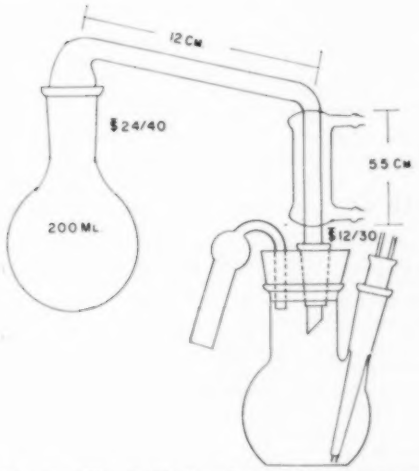

Fig. 2.—Microdistillation of water from powdered extracts.

TABLE I.—DETERMINATION OF WATER IN SOME MEDICINAL CHEMICALS

Compound	K. F. Method, %	Official Method, %	Requirement, %
Acetanilid	0.01	0.01	N. M. T. 0.5
	0.01	0.01	
Acetophenetidin	0.02	0.02	N. M. T. 0.5
	0.02	0.03	
Aminopyrine	0.03	0.02	N. M. T. 1
	0.02	0.02	
Atropine	0.23	....	.....
	0.27	....	
Atropine sulfate[a]	3.96	4.02[a]	N. M. T. 4
	3.99	3.97	
Cinchonidine sulfate	5.41	5.20	N. M. T. 8
	5.41	5.23	
Cinchonine sulfate	4.72	4.69	N. M. T. 5
	4.74	4.72	
Codeine sulfate	7.28	7.25	N. M. T. 12
	7.32	7.23	
Morphine sulfate	11.88	11.81	N. M. T. 12
	11.89	11.79	
Pilocarpine hydrochloride	0.07	0.06	N. M. T. 3
	0.08	0.04	
Quinidine sulfate	4.75	4.67	N. M. T. 5
	4.72	4.62	
Quinine hydrochloride	6.55	6.30	N. M. T. 10
	6.54	6.33	
Quinine sulfate	4.77	4.65	N. M. T. 5
	4.75	4.66	
Sulfathiazole sodium	8.86	8.82	6–9
	8.84	8.78	
Strychnine sulfate	10.07	10.02	N. M. T. 11.5
	10.12	10.09	

[a] The official drying temperature of 105° was found to be too low for atropine sulfate. A temperature of 125° was used.

DISCUSSION

From Table I it may be seen that the results obtained by the Karl Fischer method in general agree very closely with those obtained by the official drying methods. In a few cases oven drying gave too low values. Titration of the oven-dried compounds accounted for this difference (Table IV).

As shown in Table II, oven drying of powdered extracts gives inconsistent results, and it is difficult to obtain constant weights. The method, therefore, cannot be depended upon for determination of the water content of powdered extracts. The Karl Fischer method, on the other hand, appears to give reproducible values when sufficient time is allowed for complete extraction of the water. The water content of commercial powdered extracts varies over a fairly wide range and some sort of standardization is desirable. A limit value of 5% would seem appropriate.

Cold creams give too high results by direct titration with the Karl Fischer reagent. This is due to the presence of sodium tetraborate which reacts with the reagent (8).

SUMMARY

A modified Karl Fischer reagent, containing ethylene glycol monomethyl ether instead of methanol, was found to be much more stable than

TABLE II.—DETERMINATION OF WATER IN POWDERED EXTRACTS

Extract	Oven Drying 103–105°: 3 Hr.	6 Hr.	9 Hr.	Karl Fischer Titration: 15	30	60*	Toluene Microdist. + K. F. Titration
Belladonna I	3.02	4.43	4.57	3.28	3.43	3.44	3.47
	3.16	4.53	4.63	3.41	3.47	3.48	3.47
Belladonna II	4.97	5.73	5.88	5.37	5.68	5.64	5.60
	5.54	6.00	6.03	5.22	5.63	..	5.64
Nux vomica	1.71	1.87	1.97	1.99	2.05	2.06	2.06
	1.91	2.00	2.06	1.95	2.06	2.04	2.00
Licorice	4.73	4.98	4.99	5.33	5.68	5.69	..
	4.74	5.06	5.10	5.39	5.64	..	..
Cascara	7.98	8.11	8.33	8.22	8.26	8.24	8.23
	7.92	8.13	8.25	8.06	8.20	..	8.29

* Time of extraction in minutes.

TABLE III.—DETERMINATION OF WATER IN OINTMENT BASES

Preparation	K. F. Titration	Toluene Dist.
Lanolin	30.0	29.4
	29.7	29.1
Cold Cream I	23.1	21.1
	23.2	21.1
Cold Cream II	20.6	19.1
	20.5	19.5
Beeler's Base*	71.7	71.2
	71.3	71.8
"Carbowax 1500"	1.74	..
	1.78	..

* Beeler (10).

TABLE IV.—DETERMINATION OF WATER IN SOME OVEN-DRIED COMPOUNDS

Compound	K. F., %	Oven Drying, %	K. F. of Oven Dried Compounds, %
Cinchonidine sulfate	5.41	5.20	0.20
Quinine hydrochloride	6.55	6.30	0.23
Quinine sulfate	4.76	4.66	0.06

the original reagent. This modified reagent was used to determine the water content of various medicinal chemicals, powdered extracts, and ointments. Reproducible and reliable results were obtained with all compounds and preparations analyzed with the exception of cold creams. Both visual and electrometric end points were used.

A method for combined distillation and titration of water has been described.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

6-Acetamido-2-hydroxynaphthalene
Acetonitrolic acid
β-Amyrinol
Arecaidine
Arginase
Aurantia
Calcium-β,β-diglyceryl phosphate
Calcium palmityl phosphate
1,1-Bis-(*p*-chlorophenyl)ethanol
Choline stearate
Coronene
o-Cresyl-β-D-glucoside
Cupreine
Cystamine sulfoxide
Desoxyephedrine saccharinate
Dihydroquercetin
Glutaraldehydic acid
Glucamine
Luciferin
Methoxinine

A Comparison of Vitamin B_{12} Potentiators*

By GEORGE B. GRIFFENHAGEN and ERSILIA F. DeGUIA

A comparison between duodenal substance, desiccated stomach, folic acid, iron, and thymine as potentiators of vitamin B_{12} was made. Folic acid seemed to be the most active vitamin B_{12} potentiator tested. Red cell counts and hemoglobin levels of the chicks fed this combination returned to normal after fourteen days of treatment. Iron did not have a significant effect when used with the combination of folic acid and vitamin B_{12}. The combination of vitamin B_{12} and thymine produced some hemopoiesis but was not sufficient to bring the red cell counts and hemoglobin levels back to normal. Neither duodenal substance nor desiccated stomach had any potentiating effect on vitamin B_{12}.

THE HEMOPOIETIC activity of liver and liver extracts has been explained on the basis of the presence of a principle resulting from the *in vivo* interaction of an intrinsic and an extrinsic factor. As early as 1932, Walden and Clowes (1) observed that the potency of orally administered liver extract may be greatly enhanced by incubation with gastric juice or gastric mucosa. These results support the presence of an intrinsic factor as well as the antipernicious anemia principle in liver.

In 1948, Rickes, *et al.* (2), isolated a crystalline compound named vitamin B_{12} from liver which in microgram quantities produced a positive hematologic response in Addisonian pernicious anemia. Berk, *et al.* (3), later observed that an effect analogous to the potentiation effect of gastric juice on orally administered liver extract occurred when gastric juice was given with orally administered vitamin B_{12}. This fact indicates that vitamin B_{12} and the extrinsic factor are either identical or closely related substances.

In search for a source of the intrinsic factor, Bethell, *et al.* (4), reported that desiccated extracts of hog duodenal mucosa, which by themselves are without hemopoietic activity, induced remissions in patients with pernicious anemia when administered daily by mouth in combination with 5 μg. of vitamin B_{12}. Spies, *et al.* (5), likewise noted some clinical and hematologic improvement in patients with macrocytic anemia in relapse, given one capsule of the reaction product "Berubozyme" (9 μg. of vitamin B_{12} and the intrinsic factor present in 0.33 Gm. of a concentrate from hog duodenum) daily for ten days.

According to Ternberg and Eakin (6) the intrinsic factor is a protein, apoerythein, which combines specifically with vitamin B_{12} to form a tightly bound complex called erythein. Erythein is either more readily absorbed than free vitamin B_{12}, or combination of the vitamin with the protein protects it from destruction, permitting subsequent absorption.

Other drugs including folic acid, iron, and thymine have also been demonstrated to have potentiating activity toward vitamin B_{12}. Nichol, *et al.* (7), demonstrated that vitamin B_{12} combined with folic acid produced more rapid regeneration of hemoglobin in anemic chicks than treatment with a similar dosage of folic acid or vitamin B_{12} alone. In fact, Schwartz, *et al.* (8), reported that folic acid was therapeutically inadequate when used alone, while vitamin B_{12} alone did not have a significant effect on the anemia of chicks fed a folic acid and vitamin B_{12} deficient diet.

According to Meyer, *et al.* (9), vitamin B_{12} converts free folic acid to the reduced form and this latter compound is utilized in the production of the erythrocyte. Wright, *et al.* (10), suggested that the action of folic acid in pernicious anemia arises from increased thymine synthesis. Spies, *et al.* (11), demonstrated that thymine was effective when given in large doses in the treatment of nutritional macrocytic anemia, tropical sprue, and pernicious anemia.

DeGuia and Wolfred (12) observed that either vitamin B_{12} or folic acid plus iron produced better effects than either of them used alone. These effects did not surpass the one produced by the combination of folic acid and vitamin B_{12}. From these findings it is apparent that some type of comparison should be made between duodenal substance, desiccated stomach, folic acid, iron, and thymine as potentiators of vitamin B_{12}.

PROCEDURE

Day-old, pure strain, white Leghorn chicks kept in electrically heated incubators were fed the following special vitamin B_{12} deficient diet until they became anemic: casein 25 Gm. %, cerelose 50.7 Gm. %, gelatin 10 Gm. %, soybean oil *q. s.* 6.0 Gm.

* Received August 31, 1951, from the Pharmaceutical Laboratories, Nion Corporation, Los Angeles, Calif.
Presented to the Scientific Section, A. PH. A., Buffalo meeting, August, 1951.

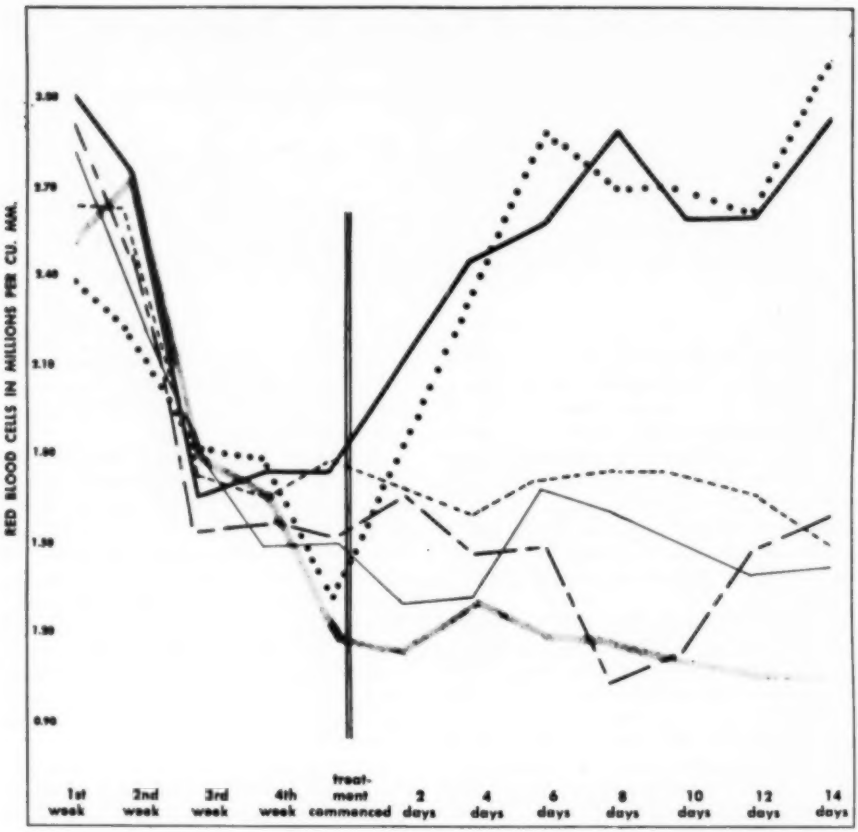

Figure 1

%, salt mixture 5.0 Gm. %,[1] methionine 0.3 Gm. %, thiamine 0.4 mg. %, riboflavin 0.8 mg. %, niacin 5 Gm. %, pyridoxine 0.6 mg. %, calcium pantothenate 2 mg. %, biotin 0.02 mg. %, choline 200 mg. %, inositol 100 mg. %, tocopherol 0.3 mg. %, vitamin K 2.5 mg. %, vitamin A 1,700 units, vitamin D 170 units.

They were leg-banded for identification and blood samples were taken by intravenous wing punctures. Red blood cell counts and hemoglobin levels were taken at weekly intervals until anemia developed. On the basis of the supplements to be administered with their diet, the chicks were divided into groups of 12 as follows:

Group I No supplement administered.
Group II Vitamin B_{12} plus folic acid and ferrous gluconate.
Group III Vitamin B_{12} plus desiccated stomach.
Group IV Vitamin B_{12} plus duodenal substance.
Group V Vitamin B_{12} plus folic acid.
Group VI Vitamin B_{12} plus thymine.

Upon treatment, blood counts and hemoglobin levels were taken at one- to two-day intervals until they returned to normal.

DISCUSSION

The normal number of red blood cells of the chicks used in these experiments ranged from 2.44 to 3.36 million per cubic millimeter of blood(Fig. 1). The normal hemoglobin level varied from 6.5 to 7.0 Gm./100 cc. of blood (Fig. 2). These results were either

[1] Salts used according to nutritional requirements, *J. Nutrition*, **32**, 460(1946).

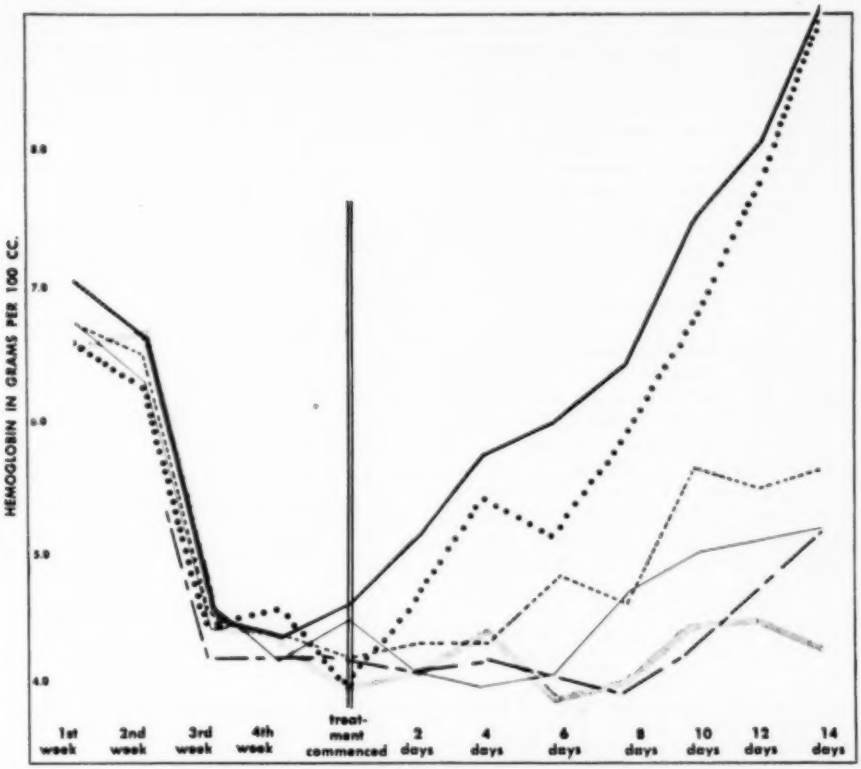

Figure 2

equal to or slightly lower than the values reported in previous literature (12).

The chicks reached the anemic levels twenty-five to thirty-three days subsequent to the administration of the basal deficient ration. Of the 72 chicks, 11 died before reaching the anemic state. This left eleven chicks in groups I and II, eight in group III, twelve in group IV, ten in group V, and nine in group VI.

Vitamin B_{12} Plus Folic Acid and Ferrous Gluconates.—Red blood cell counts and hemoglobin levels of the chicks fed a combination of 6 μg. of vitamin B_{12}, 6 mg. of folic acid, and 6 mg. of ferrous gluconate per 100 Gm. of diet, returned to normal in fourteen days. The effect of this combination was equivalent to that of folic acid plus vitamin B_{12}. The iron present in ferrous gluconate apparently had no significant potentiating effect on this combination. This combination cured the paralysis of the chicks, and normal feathering developed within five days.

Vitamin B_{12} Plus Desiccated Stomach.—Chicks fed a combination of 6 μg. of vitamin B_{12} plus 200 mg. of desiccated stomach per 100 Gm. of diet showed only slight regenerations of red blood cells and hemoglobin. The paralysis of the chicks was not cured by this combination, and feathering did not return to normal.

Vitamin B_{12} Plus Duodenal Substance.—Chicks fed 6 μg. of vitamin B_{12} plus 200 mg. of duodenal substance per 100 Gm. of diet showed only slight regenerations of red blood cells and hemoglobin comparable to that produced by vitamin B_{12} plus desiccated stomach. This combination did not cure the paralysis of the chicks, nor did feathering return to normal.

Folic Acid Plus Vitamin B_{12}.—Red blood cell counts and hemoglobin levels of chicks fed 6 mg. of folic acid plus 6 μg. of vitamin B_{12} per 100 Gm. of diet returned to normal after fourteen days of treatment. The paralysis of the chicks was cured

by this combination, and normal feathering developed within five days.

Vitamin B_{12} Plus Thymine.—Chicks fed 6 μg. of vitamin B_{12} plus 25 mg. of thymine per 100 Gm. of diet, produced more red blood cells and hemoglobin than did vitamin B_{12} plus either duodenal substance or powdered stomach. However, the regenerations were not sufficient to bring the red blood cell counts and hemoglobin levels back to normal. This combination did not cure the paralysis of the chicks, but feathering returned to normal in a week's time.

Controls.—The chicks used as controls remained anemic, had poor feathering, enlarged crops, and were all paralyzed.

SUMMARY

Folic acid seemed to be the most active vitamin B_{12} potentiator tested. Its combination with vitamin B_{12} cured the induced anemia. Red blood cell counts and hemoglobin levels of the chicks fed this combination returned to normal after fourteen days of treatment. Iron did not have a significant effect when used with the combination of folic acid and vitamin B_{12}. The combination of vitamin B_{12} and thymine produced more hemopoiesis than vitamin B_{12} plus either duodenal substance or desiccated stomach, but was not sufficient to bring the red blood cell counts and hemoglobin levels back to normal. Neither duodenal substance nor desiccated stomach had an appreciable potentiating effect on vitamin B_{12}.

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Glycine Esters of Therapeutically Useful Phenols*

By GEORGE P. HAGER, PIERRE M. ANKER,† and LEE-MING CHOW‡,§

In a search for water-soluble derivatives that might be free from various undesirable side effects, the preparation of glycine esters of diethylstilbestrol, methyl salicylate, and other therapeutically useful phenols, was undertaken. The esters were obtained as hydrochlorides by hydrogenolysis of the corresponding N-(benzyloxycarbonyl)-glycyl esters. The analgetic activity of methyl glycylsalicylate hydrochloride was tested by a variable radiant heat procedure and found to be equal to or slightly greater than that of acetylsalicylic acid under the conditions of the experiment.

THE PREPARATION of glycine esters of diethylstilbestrol, salicylic acid, methyl salicylate, and other therapeutically useful phenols, was undertaken in a study dealing with the general problem of water-insolubility and various side effects frequently encountered in the therapeutic use of phenolic substances. Glycine derivatives in which either the amino group or the carboxyl group is free are capable of forming water-soluble salts, and such derivatives are formed in the physiological detoxication of aromatic carboxylic acids, arylacrylic acids, arylacetic acids, and certain others (1). It is understandable, therefore, that glycine derivatives of physiologically active alcohols, phenols, and amines with low water-solubility, have been prepared because of the solubilizing effect of the glycine moiety. The glycine derivatives of phenetidin (aminophenacetin or phenocoll) (2), aniline (3), phenol (4), and dihydromorphine (4), have been found to possess the pharmacologic effect characteristic of the parent substance.

The hydrochlorides of phenolic glycinates can be prepared by hydrogenolysis of the corresponding phenolic N-(benzyloxycarbonyl)glycin-

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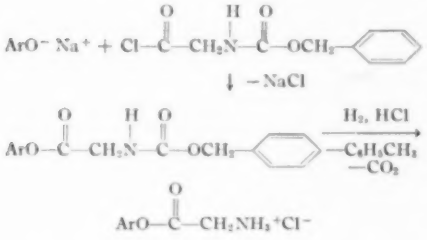
‡ Li Foundation Fellow, 1949–1951.
§ The authors wish to thank Dr. John C. Krantz, Jr., for his advice and guidance in the pharmacological portion of this investigation.

ates which result in the reaction of the phenol or its sodium salt with N-(benzyloxycarbonyl)-glycyl chloride, a reagent that is used for introduction of glycine into peptides (5).



This series of reactions was applied successfully in the preparation of diethylstilbestryl diglycinate dihydrochloride and methyl glycylsalicylate hydrochloride. Satisfactory derivatives could not be obtained from 5,7-dichloro-8-hydroxyquinoline or salicylic acid, although the glycyl ester of the latter (glycylsalicylic acid) was prepared previously by ammonolysis of chloroacetylsalicylic acid (6).

EXPERIMENTAL

All melting points are uncorrected and were observed by use of a Fisher-Johns melting point apparatus.

N-(Benzyloxycarbonyl)glycine was prepared by the reaction of benzyloxycarbonyl chloride (benzyl chloroformate or carbobenzoxy chloride) with glycine in yields of 95% according to the procedure described by Carter, *et al.* (7), when four to five times the quantities of reagents recommended were used and the reaction mixture was left standing overnight at room temperature before isolating the product.

The N-(benzyloxycarbonyl)glycine was converted to the corresponding acid chloride by treatment with phosphorous pentachloride at low temperatures (5), and was employed at once in the reaction with phenols as described below.

Diethylstilbestryl Di[N-(benzyloxycarbonyl)glycinate].—In a solution of 2.3 Gm. (0.1 equivalent) of sodium in 200 cc. of absolute ethyl alcohol, was dissolved 13.4 Gm. (0.05 mole) of diethylstilbestrol by gently refluxing for two hours. After complete removal of the ethyl alcohol by distillation under reduced pressure, the disodium salt of diethylstilbestrol was obtained as a solid and was washed with dry ether and dried.

A mixture of 15.6 Gm. (0.05 mole) of the disodium salt of diethylstilbestrol and 100 cc. of water was cooled to 0° and treated dropwise at that temperature with a solution of 22.7 Gm. (0.1 mole) of N-(benzyloxycarbonyl)glycyl chloride in 66 cc. of dry ether. During this addition the mixture was vigorously shaken, and the agitation was continued for one-half hour at 0° after addition was complete. After the mixture had stood overnight at room temperature, the slightly yellow precipitate which had formed was filtered and washed with one portion of water and two of cold alcohol to remove any diethylstilbestrol or its disodium salt. The diethylstilbestryl di[N-(benzyloxycarbonyl)glycinate] obtained in this way was recrystallized three times from ethyl alcohol, and a 16.5-Gm. (50% of theoretical) yield of a white powder, m.p. 196.5°, was obtained.

Anal.—Calcd. for $C_{38}H_{38}N_2O_8$: C, 70.14; H, 5.89; N, 4.31. Found: C, 70.40, 70.40; H, 6.08, 6.19; N, 4.44, 4.30.

Diethylstilbestryl Diglycinate Dihydrochloride.—A solution of 5 Gm. (0.0077 mole) of diethylstilbestryl di[N-(benzyloxycarbonyl)glycinate] in 1.5 L. of absolute ethyl alcohol was prepared with gentle heating, and placed in a 3-L. three-neck flask. A palladium catalyst (palladium black) prepared from 4 Gm. of palladium chloride by reduction with formaldehyde in strongly alkaline solution (8) was added, along with 16.2 cc. (5% excess) of 1 *N* alcoholic HCl. A rapid stream of hydrogen was passed into the vigorously stirred mixture at atmospheric pressure. Hydrogen was introduced in this way for one and one-half hours while the temperature of the mixture was maintained at 70°, and for an additional six hours after heating was discontinued. After filtration of the catalyst, the ethyl alcohol was removed by distillation under reduced pressure. The brown residue was dissolved in hot absolute ethyl alcohol and left standing overnight in the refrigerator. The crystals which separated from the alcohol solution were combined with an additional crop which was precipitated by careful addition of dry ethyl acetate. By this means, repeating the recrystallization from ethyl alcohol if necessary, a 1.2-Gm. (33% of theoretical) yield of a compound believed to be diethylstilbestryl diglycinate dihydrochloride, m. p. 214°, dec., was obtained. The product was very hygroscopic and decomposed on standing to form a sticky brown mass. Low carbon and nitrogen values and high hydrogen values in the following analytical data are probably a result of the high hygroscopicity of the product.

Anal.—Calcd. for $C_{22}H_{28}Cl_2N_2O_4 \cdot H_2O$: C, 55.81; H, 5.96; N, 5.92. Found: C, 55.48, 55.40; H, 6.50, 6.55; N, 5.53, 5.54.

Methyl N-(Benzyloxycarbonyl)glycylsalicylate.—A mixture of 23.3 Gm. (0.153 mole) of methyl salicylate and 153 cc. of 1 *N* sodium hydroxide was placed in a three-neck flask fitted with a dropping funnel, mechanical stirrer, and thermometer, and chilled to −10° by means of an ice-salt bath. A solution of N-(benzyloxycarbonyl)glycyl chloride, prepared from 32 Gm. (0.153 mole) of N-(benzyloxycarbonyl)glycine, in 100 cc. of cold dry ether, was added dropwise with vigorous stirring to the solution of the sodium salt of methyl salicylate. The addition was complete after one hour, and the mixture was stirred for one-half hour longer at 0°. The crystalline precipitate which separated after the reaction mixture had stood at room temperature for a short time was filtered, washed with water, and recrystallized from dilute alcohol. By this means a 16-Gm. (30.4% of theoretical) yield of methyl N-(benzyloxycarbonyl)glycylsalicylate, m. p. 82°, was obtained.

Anal.—Calcd. for $C_{18}H_{17}NO_6$: C, 62.95; H, 5.16; N, 4.27. Found: C, 62.76; H, 4.99; N, 4.10.

Methyl Glycylsalicylate Hydrochloride.—A solution of 8.44 Gm. (0.0246 mole) of methyl N-(benzyloxycarbonyl)glycylsalicylate in 100 cc. of absolute ethyl alcohol was treated with 25.8 cc. of 1 *N* alcoholic HCl or its equivalent, and 2 Gm. of a 10% palladium on carbon catalyst (9) was added. The mixture was shaken in hydrogen under 60-lb. pressure for two hours at room temperature. After filtration of the catalyst and removal of the alcohol by distillation under reduced pressure, the white crystalline residue was recrystallized repeatedly from mixtures of alcohol and ether. By this means, a 4.5-Gm. (40% of theoretical) yield of methyl glycylsalicylate hydrochloride, m. p. 175–177°, was obtained.

Anal.—Calcd. for $C_{10}H_{12}ClNO_4$: C, 48.81; H, 4.96. Found: C, 48.41; H, 5.08.

Methyl glycylsalicylate hydrochloride was found to be quite soluble in water and alcohol. The alcohol solution appeared to be stable, but the compound underwent hydrolysis rapidly in aqueous solution.

PHARMACOLOGY

The analgetic effect of methyl glycylsalicylate hydrochloride was tested by the variable radiant heat procedure of Hardy, Wolff, and Goodell as modified by Thorp (10, 11).

Pain threshold measurements were made on white rats which had been fasted for twenty-four hours before the test. An aqueous solution containing the desired dose was administered by stomach tube, and readings were made at fifteen, thirty, and sixty minutes after administration. Data were obtained which made possible a comparison of the maximum pain threshold readings in treated rats and average normal readings in the same rats before treatment. The increase in the pain threshold following administration of the analgesic was then expressed as a percentage of the normal pain threshold.

In a series of 17 rats receiving 200 mg. of methyl glycylsalicylate hydrochloride per Kg. of body weight a mean value of 20.9% (σ = 12.0, ϵ = 2.91) increase in pain threshold was observed. In a control series of 17 rats receiving 200 mg. of acetylsalicylic acid per Kg. of body weight, a mean value of 12.2% (σ = 5.3, ϵ = 1.3) increase in pain threshold was observed. The "*t*" value (12) calculated from these data was found to be 2.77. Therefore, it appears that methyl glycylsalicylate hydrochloride exerts an analgetic effect at least equal to that of acetylsalicylic acid and, perhaps, slightly higher under the conditions of this experiment.

SUMMARY

1. In a search for water-soluble derivatives of therapeutically useful phenols which might be free from various undesirable side effects, the hydrochlorides of the glycine esters of diethylstilbestrol and methyl salicylate have been prepared. Unsuccessful attempts were made in this investigation to prepare similar derivatives of acetylsalicylic acid and 5,7-dichloro-8-hydroxyquinoline.

2. Hygroscopicity and instability, especially to hydrolysis, made the preparation of pure derivatives of this type rather difficult, and hindered pharmacologic studies which had been planned as part of this investigation.

3. Methyl glycylsalicylate hydrochloride appeared to exert an analgetic effect at least equal to and possibly slightly higher than that of acetylsalicylic acid in pain threshold tests of the variable radiant heat type.

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Pressor Activity of *l*- and *d*-Norepinephrine, U. S. P. Reference Standard Epinephrine, and Synthetic *l*-Epinephrine*

By EDWARD E. SWANSON and K. K. CHEN†

l-Norepinephrine has a higher pressor activity than the *d*-isomer, in the ratio of 46:1 in pithed dogs, and 45:1 in pithed cats. There is no demonstrable difference in the pressor activity of U. S. P. reference standard epinephrine, *l*-norepinephrine, and synthetic *l*-epinephrine in pithed dogs. U. S. P. reference standard epinephrine has about the same pressor activity as synthetic *l*-epinephrine in pithed cats, but it has a lower potency than *l*-norepinephrine in the same preparation. Synthetic *l*-epinephrine in phenobarbitalized or etherized dogs is less active than *l*-norepinephrine, but more active in phenobarbitalized or etherized cats.

FOLLOWING the resolution of epinephrine into its optical isomers (1), pharmacological investigation at one time or another has shown that the *l*-form is 15 to 20 times as active as the *d*-isomer in raising blood pressure recorded by various methods (2–6). Indeed, this is generally true with other aromatic amines, namely, the *l*-isomer is more active than the *d*-isomer (7). When norepinephrine was resolved into its optical isomers by Tullar (8), it was possible to show that according to the U. S. P. dog pressor assay the *l*-isomer was 27 times as potent as the *d*-form (9). In the same series of experiments Luduena, *et al.*, reported that *l*-norepinephrine had 1.6 times the pressor action of U. S. P. reference standard epinephrine (9).

Because of the long experience in this laboratory with pithed cats and dogs in the study of pressor amines according to the procedure originally described by Elliot (10), we undertook the comparison of *l*- and *d*-norepinephrine, synthetic *l*-epinephrine, and U. S. P. reference standard epinephrine. The last-mentioned product has recently been shown to contain up to 18 per cent *l*-norepinephrine (11). Our experiments in the present work were carried out in both pithed cats and dogs. For checking purposes anesthetized dogs and cats (phenobarbital or ether) were also used. Sodium phenobarbital was intravenously injected, 125 mg./Kg. for the cat, and 150 mg./-Kg. for the dog. A few animals required a supplementary dose amounting to 10–20 per cent of the initial dose. The optical isomers of norepinephrine and synthetic *l*-epinephrine were in the form of the bitartrate salt. *l*-Norepinephrine bitartrate had 1 molecule of water of crystallization, whereas the other two products were in anhydrous form. The U. S. P. epinephrine was mixed with a theoretical amount of *d*-tartaric acid and made into solution. For comparison in pressor evaluation the molecular equivalent solution of each compound was prepared. Molar solutions of 0.0005 were employed for *l*-norepinephrine, *l*-epinephrine, and U. S. P. reference standard epinephrine. For *d*-norepinephrine a molar solution of 0.05 was required. Doses varied according to the activity of the compound and the sensitivity of the animal. All solutions were injected intravenously. Thirty dogs were used, their weights ranging from 4.2 to 12.8 Kg. with an average of 7.6 Kg. Thirty-one cats were studied, their weights varying from 1.9 to 3.8 Kg. with an average of 2.5 Kg. Each derivative was tested with each procedure in five or more animals. The doses were all measured as the bitartrates. The geometric mean with its standard error in milligrams was calculated for each compound.

RESULTS AND DISCUSSION

Pithed Dogs and Cats.—Table I shows that the pressor activity of synthetic *l*-epinephrine is identical with that of U. S. P. reference standard epinephrine in both species. Similarly in the pithed dog there is no significant difference between the pressor action of *l*-norepinephrine and U. S. P. reference standard epinephrine. In the pithed cat, however, U. S. P. epinephrine was more potent than *l*-norepinephrine, for 1 mg. of the latter was equivalent to a mean of 0.7122 mg. of U. S. P. reference standard epinephrine in seven tests. This is of interest in view of the reports that in urethanized cats (12) racemic norepinephrine is one-third more active than racemic epinephrine.

In agreement with Luduena, we observed that *d*-norepinephrine had a much lower pressor activity than the *l*-isomer. The two optical isomers of nor-

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TABLE I.—PITHED DOGS AND CATS

Compound	Species of Animal	No. of Animals	U. S. P. Epinephrine Equivalent to 1 Mg. of Amine in Comparison, Geometric Mean ± S. E., Mg.
Synthetic *l*-epinephrine	Dogs	5	1.00
	Cats	5	1.00
l-Norepinephrine	Dogs	6	0.956 ± 0.097
	Cats	7	0.7122 ± 0.0532

epinephrine when directly compared in the same preparations have pressor ratio, $l:d = 46:1$ for dogs and 45:1 for cats (Table II)—a remarkably close check. Luduena, *et al.* (9), reported a ratio of 27:1 in phenobarbitalized dogs.

TABLE II.—PITHED DOGS AND CATS

Compound	Species of Animal	No. of Animals	*l*-Norepinephrine Equivalent to 1 Mg. of the *d*-Form, Geometric Mean ± S. E., Mg.
d-Norepinephrine	Dogs	6	0.0216 ± 0.0028
	Cats	5	0.0221 ± 0.0033

Phenobarbitalized or Etherized Dogs and Cats.—As shown above, results in our pithed animals did not confirm the work of Luduena, *et al.* (9). Their data indicated that *l*-norepinephrine was more potent than U. S. P. reference standard epinephrine in phenobarbitalized dogs, whereas in our pithed dogs the pressor activity of *l*-norepinephrine, U. S. P. reference standard epinephrine, and synthetic *l*-epinephrine may be considered the same. Table III

TABLE III.—ANESTHETIZED DOGS AND CATS

Compound	Species of Animal	No. of Animals	Kind of Anesthetic Used	Synthetic *l*-Epinephrine Equivalent to 1 Mg. of *l*-Norepinephrine, Geometric Mean ± S. E., Mg.
l-Norepinephrine	Dogs	8	Phenobarbital sodium	0.930 ± 0.0225
		5	Ether	0.862 ± 0.0698
	Cats	8	Phenobarbital sodium	1.219 ± 0.107
		6	Ether	1.223 ± 0.107

shows that in both phenobarbitalized and etherized dogs synthetic *l*-epinephrine has a tendency to be more active than *l*-norepinephrine. The difference is statistically significant at the 5% level because it exceeds two standard errors. It must be mentioned that our test differed from the U. S. P. procedure employed by Luduena, *et al.* (9), in their experiments. Their dogs were atropinized prior to the administration of the pressor substance. In our tests the dogs were simply anesthetized and set up for blood pressure recording in the usual manner.

In the phenobarbitalized or etherized cats, however, there is evidence that *l*-norepinephrine is more active than synthetic *l*-epinephrine. The difference is also statistically significant at the 5% level. In this respect our results agree with those of Luduena, *et al.* (9), in dogs and with those of Tainter (12) using racemic norepinephrine and epinephrine in anesthetized cats. It cannot be overemphasized that the anesthetic agent on the one hand and species difference of susceptibility on the other are frequent sources of discrepancy of results.

SUMMARY

1. In the pithed dog the pressor activity of *l*-norepinephrine is approximately the same as that of synthetic *l*-epinephrine and U. S. P. reference standard epinephrine. No difference can be demonstrated between U. S. P. epinephrine and *l*-epinephrine in the pithed cat.

2. In the pithed cat *l*-norepinephrine is less active than U. S. P. reference standard epinephrine.

3. The pressor ratio of activity between *d*- and *l*-norepinephrine is 1:46 in the pithed dog, and 1:45 in the pithed cat.

4. In the phenobarbitalized or etherized dog *l*-norepinephrine is less active than synthetic *l*-epinephrine. In contrast, *l*-norepinephrine is more potent than synthetic *l*-epinephrine in the phenobarbitalized or etherized cat.

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The Effects of Contaminants on the Properties of Medicinal Kaolins*

By A. HALPERN, A. J. MONTEBOVI, J. V. POWERS, C. H. BRADNEY, A. B. ANDERSON, and R. J. CHASE

Properties of kaolin were critically studied from the viewpoint of proposing a more stringent set of standards in order to better define this pharmaceutical product. Determinations as to chemical composition, settling rate, and absorption of alkaloids and dyes were carried out. Microphotographs of the contaminants present were obtained and an effort was made to relate the presence of these contaminants with the observed pharmaceutical activity.

In 1936 Mutch (1) demonstrated that the official British Pharmacopoeia tests for kaolin were insufficient to establish either the identity, chemical purity, pharmacological activity, or freedom from insoluble gritty contaminants. Recently it was reported (2) that marked variation occurs among samples of kaolin which meet the National Formulary requirements. This complacence toward the standards of kaolin and the resultant acceptance of a product on the basis of limited purity requirements becomes much more significant when we recognize its widespread use in internal preparations.

Kaolin is a decomposition product from the weathering of granite and other rocks. The term is generic and includes many closely related products which vary with the geological area. Chemically, kaolin is $Al_2O_3.2SiO_2.2H_2O$, but not all hydrated aluminum silicates may be called "kaolin." It has been established that at least three different groups of china clay may be identified as kaolin (3). These are kaolinite, dickite, and nacrite, all having the same chemical composition.

The superior medicinal kaolins are derived from the kaolinite group obtained from England and the United States. The crude material is purified by elutriation which removes only the larger particles of quartz, mica, and partially altered feldspar. These clays still contain much gritty material which can be detected readily by grinding between the teeth. In fact, on simple macroscopic examination crystals of quartz, tourmaline, feldspar, and mica can be seen throughout the predominant kaolin. It is noted that the official description of kaolin is "a native aluminum silicate, powdered and freed from gritty particles by elutriation" (4).

In order to obtain a colloidal kaolin, a peptizing process is utilized which involves suspending the clay in an electrolyte solution. An electric charge is imparted upon the surface of the clay which permits it to remain suspended while the heavier grit settles. The colloidal clay is then flocculated by the addition of another electrolyte or by means of an electric field. When this material is collected and dried it is substantially free of grit. The method of flocculation is important to characteristics of the final product. All kaolins purified by wet-chemical processes are contaminated by adsorbed electrolytes. The degree of contamination varies with the concentration of electrolyte used and the duration of contact. Obviously, such contamination is absent in products obtained through an electro-precipitation process. It is of interest to note that kaolin purified in the latter manner is readily available.

The present study was initiated to investigate the effects of the different methods of purification on the therapeutic properties of the kaolins. The adsorption capacity is an important factor in the rationale of employing kaolin for an internal use. Particle size is a prime determinant in the over-all phenomenon of adsorption, and the method of purification was reported (5) to exert a profound effect on this property of kaolin. Rae (6) found very wide differences among the adsorptive properties of various kaolins. While Muller (7) reported that German kaolin (Merck) would remove only 50 per cent of "mussel poison" from aqueous solution, Mutch (1) showed that an English kaolin removed the toxic substances completely.

The literature has also reported (8–12) occurrence of granulomae attributable to the silica contaminants present in some kaolins. Silica (SiO_2) produces the typical and well-known lesion of silicosis, but certain silicates are also capable of producing similar reactions. Kettle (13) produced lesions using kaolin, but on analysis of his kaolin samples, they were shown to contain particles of quartz and numerous fibers of sericite (potassium aluminum silicate). Whether it is the quartz or the sericite in the kaolin which is re-

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sponsible for the lesions is still not established (14). It is of interest to note that Kettle (13) states that "with different forms of crystalline silica and with certain silicates essentially the same kind of lesion is produced, such modifications as occur depend upon the size of the silica particles and their degree of solubility." Gardner (15) concurs that the size of the particle is very important both to the type and the extensiveness of the lesion. While the greater number of the reports of granuloma formation deal with nongastrointestinal lesions, the occurrence of a gastric granuloma has also been observed (12). In the latter instance as well, silica contaminants were postulated as the etiological factor.

In view of the above information, it was deemed important to evaluate experimentally the different kaolins in order to arrive at a basis for a critical control of the possible contaminants.

EXPERIMENTAL

Representative samples of kaolin (Table I) were obtained commercially. Products originating from different sources were selected and only those described as being of medicinal grade were selected.

The theoretical formula for kaolin established the relationship between the silicon and aluminum as 1:1.04. The different kaolins were analyzed for aluminum and silicon content and their ratios computed (Table II). Mica and silica contamination would not be conclusively established by such elementary analysis, as their constituents would be present in the values already determined. However, gross adulteration would be readily apparent. A more sensitive criteria of the presence of extraneous mineral matter is the quantity of calcium and magnesium contained in the sample. The samples were also analyzed for calcium and magnesium content (Table II). It is significant that only one sample (No. 3) contained less than 0.5% of either element.

TABLE II.—CHEMICAL ANALYSIS OF THE KAOLINS

Sample No.	Per Cent				Ratio of Si:Al[a]
	Al_2O_3	SiO_2	CaO	MgO	
1	37.46	45.30	1.31	0.61	1.06
2	36.16	46.92	1.22	0.51	1.14
3	38.15	44.70	0.50	0.35	1.03
4	35.84	46.79	0.81	1.31	1.16

[a] The theoretical ratio of silicon to aluminum, calculated from the formula, $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$, is 1:1.04.

The remainder contained large proportions of either calcium or magnesium, with some as high as 1.24%.

All of the clays were peptized with a 1% sodium pyrophosphate solution. A gritty deposit quickly settled which was collected, washed, and examined microscopically (Fig. 1). The comparative purities are rather conspicuous. The sediment obtained from the kaolin prepared by an electro-flocculation procedure consists chiefly of thin small flat particles. The sediment resulting from the clays obtained by the chemical purification technique contains many angular particles of quartz as well as crystalline particles of the electrolyte used in the peptizing process. It is of interest to note the absence of the coarser contaminants in the electro-purified kaolin.

The sedimentation rate was established for the different test samples by measuring the clear zone formed on standing. To a graduate cylinder was added 100 cc. of a 5% aqueous suspension of kaolin and the graduate sealed to prevent evaporation. The sedimentation was measured periodically. The results of this study are presented in Fig. 2. It is significant that only two of the kaolins studied formed suspensions that did not settle appreciably during the seven-day observation. Two of the samples settled out within six hours. The latter samples formed plastic masses on admixture with water. These sticky coagulums would be very conducive to intestinal concretions.

It is plausible to expect a similar behavior in physiological media so that a rapid settling of the kaolin in the stomach and small intestines would result when these products were used internally.

TABLE I.—PURITY RUBRIC OF THE TEST KAOLINS

Test	Kaolin 1	Kaolin 2	Kaolin 3	Kaolin 4
Grade	Cosmetic	N. F.	Osmo—1	N. F.
Standards	Br. Pharm. and N. F. IX	Br. Pharm. and N. F. IX	Br. Pharm. and N. F. IX	Br. Pharm. and N. F. IX
Method of activation	Electrical	Chemical	Electrical	Chemical
Color	Brown Tint	Darker Brown	Light Brown	Brown
Appearance	Fine Powder	Powder	Fine Powder	Fine Powder
Odor	Nil	Slight	Nil	Slight
Taste	Earthy	Earthy	Earthy	Earthy
Feel	Silky	Silky	Silky	Silky
Lead limit	Less than 5 p. p. m.	Less than 5 p. p. m.	Less than 5 p. p. m.	Less than 5 p. p. m.
Arsenic limit	Less than 2 p. p. m.	Less than 2 p. p. m.	Less than 2 p. p. m.	Less than 2 p. p. m.
Carbonates	Nil	Nil	Nil	Nil
Soly. in 0.5 *N* HCl[a]	0.6%	0.8%	0.4%	0.6%
Methylene blue absorption[b]	9–10 cc.	7–8 cc.	13–14 cc.	8–9 cc.
Bulk[c]	1 in 34	1 in 26	1 in 55	1 in 30
Loss on ignition	12.41%	14.31%	12.20%	13.46%

[a] Based on a 1-Gm. sample. The N. F. IX requirements are "not more than 1 per cent (10 mg.)."
[b] Absorption in cc. of a 0.2% methylene blue solution per Gm. of kaolin.
[c] The volume in fluid ounces occupied by 1 lb. of the test sample.

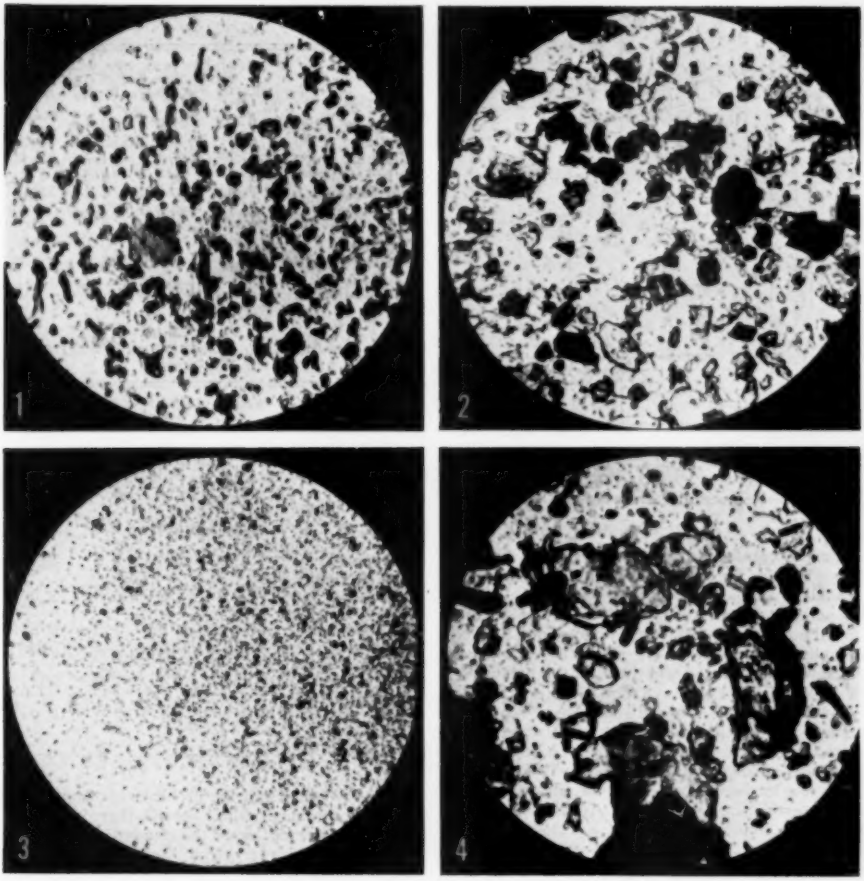

Fig. 1.—Microscopic examination of sediment at 200 magnification.

In order to establish the behavior of the different kaolins in physiological media, the sedimentation rates were re-evaluated using artificial gastric and intestinal juices (16). The procedure was identical to that described above except that the suspensions were prepared with the artificial juices rather than distilled water and the period of observation was three days. The results of this study are essentially the same as those reported for distilled water. This is rather significant since the high sedimentation rate observed in the laboratory may be translated into a poor biological activity. It is just this type of sedimentation that may be responsible for the difficulties observed with some kaolins.

It is recognized that suspending agents are incorporated into some kaolin suspensions. The use of these suspending agents does not change the basis for the rejection of kaolins with a high sedimentation rate since the surface-active compound does not alter the particle size or the degree of contamination present. The reason for a rapid settling rate is a high percentage of particles greater than 10 μ in size and a minimum of particles less than 3 μ. The British Pharmacopoeia includes a particle size limit as well as a coarse particle test. Both of these determinations are absent from the purity rubric of the National Formulary IX.

The particle size limit tests of the British Pharmacopoeia were applied to the different kaolins and the results are reported in Table III. Sample 3, which showed the least degree of contamination on microscopic analysis, was the most satisfactory from the point of view of particle size content. On the basis of these tests, three of the kaolins appear to have an abnormally high content of larger particles which would make these preparations unsuitable for internal use.

The adsorption capacity of the different kaolins for various dyes and poisons was also determined. In the experiments involving dyes, the method of the

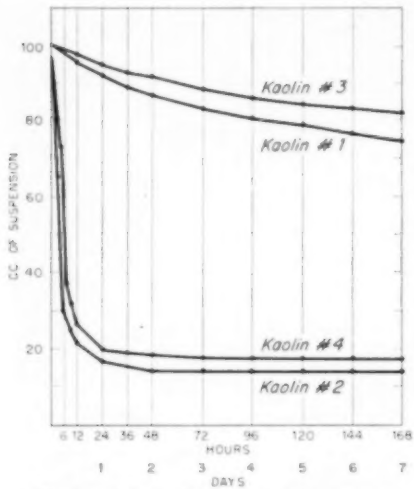

Fig. 2.—Sedimentation rates of the kaolins.

National Formulary (4) was employed. In the case of the alkaloids the following technique was utilized: To a 50-cc. standard solution of 0.1% of the alkaloid was added small weighed quantities of the kaolin. The mixture was stirred for ten minutes and 3 cc. of supernatant fluid carefully removed. The 3-cc. sample was centrifuged and the clear fluid transferred to a clean test tube. A few drops of Millon's reagent were added; if free alkaloid was shown to be present, more of the kaolin was added to the alkaloidal mixture and the process repeated. Since the kaolin was added in increments of 0.1 Gm., a good degree of reproducibility of the results was obtained. Each determination was done in triplicate and repeated after an interval of one week. The results reported in Tables IV and V are the mean observations of six experiments.

DISCUSSION

In reviewing the abundance of the reports of the behavior of the kaolins in the literature, one is impressed by two conspicuously recurring concepts. First, that the kaolins of commerce show extreme variation not only from product to product, but also from batch to batch. This is probably the basis for most of the manufacturing problems involved with products containing kaolin. Second, there is a widespread complacence toward the standards for kaolin. The National Formulary requirements have not been changed in many years. There are no methods for either determining that the product is substantially free of grit as it is defined or for limiting the particle size of the clay. The present study serves to emphasize the need for these procedures.

The method of purification must be critically reviewed before a kaolin is used for a pharmaceutical preparation. The electro-precipitation technique is obviously one of choice since there is no possibility of contamination due to the flocculating agent. A kaolin prepared in this manner is much more uniform in both particle size and resultant adsorption capacities.

It might be considered to limit the amount of contamination of foreign elements such as calcium and magnesium and also to establish a tolerance derived from the silicon-aluminum ratios. This tolerance, however, would not exclude mica and feldspar adulteration.

This investigation established kaolin 3 (osmokaolin) to be the product of choice in all of the comparative tests conducted. It most closely approximates the ideal kaolin in chemical composition, freedom of adulteration, and pharmaceutical properties.

TABLE III.—PARTICLE SIZE STUDY OF THE KAOLINS

Sample No.	Coarse Particles — Residue[a] — Gm.	%	Sp. Gr. 20/20 — 20 Min.[b]	3½ Hr.[c]
1	0.040	0.8	1.0110	1.0091
2	0.065	1.3	1.0101	1.0084
3	0.020	0.4	1.0117	1.0100
4	0.045	0.9	1.0102	1.0086

[a] The coarse particle limit of the British Pharmacopoeia is not more than 0.025 Gm. (0.5%) based on a 5-Gm. sample.
[b] A sample having a specific gravity of not less than 1.0115 corresponds to a preparation having not more than 5% of the particles larger than 10 μ in diameter.
[c] A sample having a specific gravity of not less than 1.0091 corresponds to a preparation having not more than 25% of the particles larger than 3 μ in diameter.

TABLE IV.—DYE ADSORPTION CAPACITY OF THE KAOLINS[a]

Dye	Adsorption in cc. of a 0.2% Dye Solution per Gm. of Kaolin — Kaolin 1	Kaolin 2	Kaolin 3	Kaolin 4
Methylene blue	9–10	8–9	13–14	7–8
Safranin	6–7	8–9	12–13	4–5
Malachite green	1–2	3–4	4–5	2–3
Methyl violet	1–2	3–4	4–5	2–3
Basic fuchsin	7–8	7–8	13–14	5–6
Acid fuchsin	2–3	4–5	7–8	2–3
Eosin	2–3	4–5	6–7	1–2
Congo red	5–6	7–8	10–12	5–6
Mercurochrome	3–4	3–4	6–7	2–3

[a] The data are expressed as a range to obviate fractional values.

TABLE V.—ALKALOID ADSORPTION CAPACITY OF THE KAOLINS

Agent	Weight of Alkaloid Removed by 1 Gm. of Kaolin — Kaolin 1, Mg.	Kaolin 2, Mg.	Kaolin 3, Mg.	Kaolin 4, Mg.
Atropine sulfate	5.2	3.3	9.1	4.5
Brucine hydrochloride	4.3	2.1	6.7	2.1
Colchicine salicylate	2.4	1.0	3.1	0.5
Epinephrine hydrochloride	4.1	2.6	5.7	3.1
Muscarine hydrochloride	2.1	3.2	6.3	2.0
Nicotine sulfate	1.6	0.2	1.5	0.2
Pilocarpine hydrochloride	1.2	0.5	2.4	0.2
Scopolamine hydrobromide	4.6	3.4	7.3	3.3
Strychnine hydrochloride	3.7	4.1	7.0	3.2

SUMMARY AND CONCLUSIONS

1. Physical and chemical properties of a series of kaolins were investigated.

2. Wide variations among the different samples were observed even though all are described as meeting the N. F. requirements.

3. Much of this variation appears to be due to the gross contaminants present, as demonstrated by particle size determinations and microscopic examinations.

4. On the basis of the results of this study the most satisfactory kaolin appears to be the one prepared by an electro-precipitation technique which afforded a uniformly higher index in all of the tests conducted.

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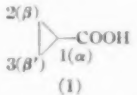
Basic Esters of Cyclopropanecarboxylic Acids*

By GEORGE P. HAGER and CHARLES I. SMITH†

A number of important musculotropic spasmolytic agents are esters of aminoalcohols and arylaliphatic acids, and their activity appears to depend to some degree on the number and complexity of the aryl groups or related hydroaromatic groups in the acid moiety. Cyclopropanecarboxylic acid can be modified by substitution with aryl groups to produce a greater variety of compounds than is possible by similar substitution of the more frequently encountered acyclic aliphatic acids. Several cyclopropanecarboxylic acids have been prepared by the reaction of ethyl diazoacetate with the appropriate hydrocarbon or diphenyldiazomethane with ethyl cinnamate, and converted to aminoalkyl esters in the usual way. By such reactions the following esters have been prepared: diethylaminoethyl 2,2-diphenylcyclopropanecarboxylate; diethylaminoethyl 2,3-diphenylcyclopropanecarboxylate; diethylaminoethyl 2,2,3-triphenylcyclopropanecarboxylate; and diethylaminoethyl dibenzonorcaradienecarboxylate.

CHOLINERGIC-BLOCKING activity, manifesting itself in various ways pharmacodynamically, is found in the basic esters of arylaliphatic acids. In many of the more active and useful compounds of this type, more than one aryl or hydroaromatic grouping or a condensed polynuclear grouping are found; and the *alpha*- or *beta*-carbons of the aliphatic acid moiety are generally involved as part of the ring systems or as points for attachment of the cyclic substituents. As examples of compounds in which these structural features are present, the basic esters of the following acids might be cited: 2-phenyl-3-hydroxypropionic acid (tropic acid), diphenylacetic acid, diphenylglycolic acid (benzilic acid), fluorene-9-carboxylic acid, xanthene-9-carboxylic acid, etc. The arylcycloalkanecarboxylic acids differ from the others in that more positions in the neighborhood of the carboxyl group are capable of bearing aryl or hydroaromatic substituents. Cyclopropanecarboxylic acid (I), like the other cycloalkanecarboxylic acids, has two positions corresponding to the *beta*-position of an acyclic carboxylic acid, which can be substituted.



(I)

A number of 1-phenylcycloalkanecarboxylic acid esters have been studied and the potential clinical usefulness of the class is illustrated by 2-diethylaminoethyl 1-phenyl-1-cyclopentanecarboxylate or Panpartit (II).

(II)

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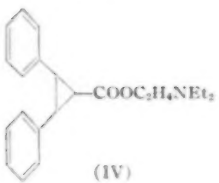
Esters of 1-phenylcyclopropanecarboxylic acid and 2-phenyl-2-methylcyclopropanecarboxylic acid are included in series of compounds of this type reported by Weston (1) and by Tilford and co-workers (2). Certain members of these series were found to have substantial neurotropic as well as musculotropic spasmolytic activity.

Because of the activity of monophenylcycloalkanecarboxylates and accepting the possibility that greater activity is found in esters of aliphatic acids bearing more than one phenyl or hydroaromatic substituent or a complex polynuclear substituent, a series of di- and triphenylcyclopropanecarboxylates (III–V) and a dibenzonorcaradienecarboxylate (VI) has been prepared. The preparation of 2-diethylaminoethyl 2,3-diphenylcyclopropanecarboxylate hydrochloride (IV) was reported by Burger and co-workers (3) while this work was in progress.

(III)



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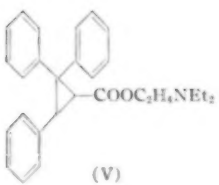

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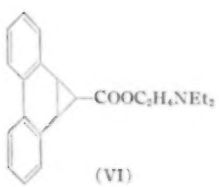
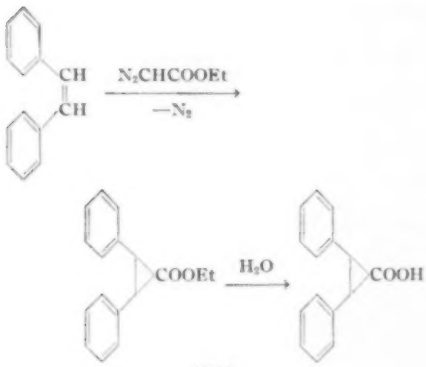


(VI)

A comparison of the pharmacologic properties of IV and VI might prove especially interesting since the latter has the form of a condensed polynuclear analog of IV. The structure of VI has the same relationship to that of IV that exists between the structures of diethylaminoethyl fluorene-9-carboxylate (Pavatrine) and diethylaminoethyl diphenylacetate (Trasentin) and which has been termed "bio-isosteric" by Burger (4).

The intermediate acids required for preparation of III, IV, and VI were prepared by reaction of ethyl diazoacetate with 1,1-diphenylethylene, *trans*-stilbene, and phenanthrene, respectively. Previously reported methods for carrying out this reaction were employed for preparation of 2, 2-diphenylcyclopropanecarboxylic acid (5) and dibenzonorcaradienecarboxylic acid (6). Because of the successful use of copper as catalyst in the reaction of ethyl diazoacetate with benzene (7) and various vinyl ethers and esters (8, 9), the effect of copper on the reaction of ethyl diazoacetate with phenanthrene, for preparation of dibenzonorcaradienecarboxylic acid, was investigated. Although comparison of yields in preparation of this compound may be somewhat misleading because of the rather wasteful purification steps that must be taken, in this investigation the yield of pure product was about doubled by use of copper powder as a catalyst. It was also found possible to replace part of the excess hydrocarbon present as solvent for the reaction by an inert solvent, cetane, without detriment to the yield. In fact, slightly better yields of pure product were obtained in several preparations of this compound when the inert solvent was employed.

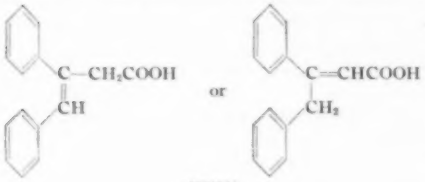
Although Buchner (10) reported an unsuccessful attempt to prepare an addition product by reaction of diazoacetic ester with styrene or stilbene, 2-phenylcyclopropanecarboxylic acid has since been prepared by reaction of diazoacetic ester with styrene (11, 12), and 2,3-diphenylcyclopropanecarboxylic acid (VII) was prepared in this investigation by reaction of diazoacetic ester with *trans*-stilbene as indicated by the following equation:



(VII)

The product had properties that corresponded to those of the racemic 2,3-diphenylcyclopropanecarboxylic acid obtained from *trans*-stilbene by Burger (3) by treatment with diazoacetic ester in a similar way.

The neutral equivalent and elementary analysis of the product were consistent with the reaction of one mole of ethyl diazoacetate with each mole of stilbene. The absence of water-soluble acids of the phthalic acid type in the oxidation products of the compound indicated that the ethyl diazoacetate did not react with carbon atoms of the cycles and must have reacted at the extranuclear double bond. Such a conclusion was confirmed by the recovery of an apparently good yield of benzoic acid after oxidation. The possibility that the product might have an isomeric structure, VIII, was also considered. However,



(VIII)

the fact that the product was unaffected by alkaline permanganate or by a solution of bromine in carbon tetrachloride at room temperature was consistent with the assigned structure, *viz.*, 2,3-diphenylcyclopropanecarboxylic acid.

2,2,3-Triphenylcyclopropanecarboxylic acid was prepared by reaction of diphenyldiazomethane with ethyl cinnamate by use of a known method (13).

The 2-diethylaminoethyl esters of the acids (III–VI) were prepared by reaction of 2-chloroethyldiethylamine with the sodium salts of the acids in isopropyl alcohol. Preliminary pharmacologic data indicate that these compounds have spasmolytic properties.[1]

EXPERIMENTAL

All melting points are corrected. Nitrogen analyses were carried out by the Hengar technique for the Kjeldahl procedure. Ethyl diazoacetate was prepared by diazotization of glycine ethyl ester hydrochloride (14). 2-Chloroethyldiethylamine was prepared from 2-diethylaminoethanol by reaction with thionyl chloride in chloroform solution in the usual way.

2, 2 - Diphenylcyclopropanecarboxylic Acid.—A 12% yield of this compound was obtained by reaction of ethyl diazoacetate with 1,1-diphenylethylene according to the directions of Wieland and Probst (5).

2, 3 - Diphenylcyclopropanecarboxylic Acid.—Ethyl diazoacetate (25 Gm., 0.22 mole) was added dropwise to 90 Gm. (0.5 mole) of *trans*-stilbene while stirring at 150° during three hours, and 0.185 mole of nitrogen was collected. The reaction mixture was poured while still hot into a 2-L. Florence flask and dissolved in 1 L. of hot absolute ethyl alcohol. To the solution, 20 Gm. of sodium hydroxide was added and the mixture was refluxed for five hours to saponify the ester. After removal of the alcohol by distillation under reduced pressure, the residue was extracted several times with hot water. The combined extract was filtered hot in order to remove unchanged stilbene, and the filtrate was acidified with concentrated hydrochloric acid. A small amount of the sodium salt of the acid generally precipitated before acidification. After standing for one hour, the precipitated acid was filtered, allowed to stand for several days, dried, and powdered.

The crude product was dissolved in slightly more than the calculated amount of 10% sodium hydroxide with the aid of heat and the solution was diluted with an equal volume of hot water. On chilling, the slightly soluble sodium salt precipitated in the form of light yellow crystals. The salt was redissolved in water and, after treatment of the solution with Norit, the acid was precipitated by addition of dilute hydrochloric acid. By this means, a 4-Gm. (9% of theoretical) yield of 2,3-diphenylcyclopropanecarboxylic acid, fine white crystals, m. p. 157 to 157.8°, reported m. p. 153–154° (3), was obtained. The product was recrystallized from a mixture of petroleum ether and carbon tetrachloride when necessary.

Neutral Equivalent.—Calcd. for $C_{16}H_{14}O_2$: 238.3. Found: 241.6.

Anal.—Calcd. for $C_{16}H_{14}O_2$: C, 80.65; H, 5.92. Found: C, 80.34, 80.29; H, 5.98, 5.91.

A sample of the sodium salt of the purified acid had no effect on cold alkaline potassium permanganate, but reduced the permanganate when heated on a steam bath for two hours. After treatment of the reaction mixture with sodium sulfite and sulfuric acid, a considerable amount of benzoic acid, as well as some unchanged 2,3-diphenylcyclopropanecarboxylic acid, precipitated and was removed completely by extraction with chloroform. The residue obtained by evaporation of the remaining aqueous solution did not char on ignition and gave negative tests for phthalic acid (15) and terephthalic acid (16). A sample of the 2,3-diphenylcyclopropanecarboxylic acid, which was slightly soluble in carbon tetrachloride, did not decolorize a solution of bromine in carbon tetrachloride.

2,2,3-Triphenylcyclopropanecarboxylic Acid.—A 42% yield of 2,2,3-triphenylcyclopropanecarboxylic acid was obtained by treatment of ethyl cinnamate with diphenyldiazomethane and saponification of the ethyl 2,2,3-triphenylcyclopropanecarboxylate which was formed according to the directions of Staudinger and co-workers (13).

Dibenzonorcaradienecarboxylic Acid.—A 3.3% yield of pure dibenzonorcaradienecarboxylic acid was obtained by reaction of ethyl diazoacetate with phenanthrene according to the directions of Drake and Sweeney (6).

[1] Pharmacologic studies are being carried out by Bristol Laboratories, Inc., and will be published elsewhere.

When the same procedure was followed, using 15 Gm. of powdered copper along with the recommended proportions of the other reactants, *viz.*, 110 Gm. (0.62 mole) of phenanthrene and 19.7 Gm. (0.17 mole) of ethyl diazoacetate, 15 Gm. of crude product was obtained. After purification by washing with cold dioxane and recrystallizing the residue from dioxane, 2.1 Gm. (6.2% of theoretical based on the amount of nitrogen evolved) of dibenzonorcaradienecarboxylic acid, m. p. 257–258°, reported m. p. 257.5 to 258° (6), was obtained. An additional 0.6-Gm. crop was recovered from the mother liquor and the actual yield was therefore somewhat higher.

In another series of experiments, part of the excess phenanthrene present for solvent purposes was replaced by cetane. In a typical run, a mixture of 90 Gm. (0.5 mole) of phenanthrene and 90 Gm. of cetane was treated with 24 Gm. (0.21 mole) of ethyl diazoacetate, and 0.167 mole of nitrogen was collected. After saponification and purification in the usual way, 1.55 Gm. (4.0% of theoretical based on the amount of nitrogen evolved) of dibenzonorcaradienecarboxylic acid, m. p. 257–258°, was obtained.

2-Diethylaminoethyl 2,2,3-Triphenylcyclopropanecarboxylate Hydrochloride.—The dry sodium salt prepared from 3.2 Gm. (0.01 mole) of 2,2,3-triphenylcyclopropanecarboxylic acid was heated under reflux with 1.36 Gm. (0.01 mole) of 2-chloroethyldiethylamine in 50 cc. of isopropyl alcohol for six hours. The isopropyl alcohol and any excess 2-chloroethyldiethylamine were removed completely by distillation under reduced pressure. The residue was extracted with four 25-cc. portions of anhydrous ether, and the combined extract was filtered. After treatment of the ether solution with dry hydrogen chloride and storage overnight at room temperature, the ether was removed by decantation or distillation. The residue was crystallized from a mixture of absolute ethyl alcohol and isopropyl ether. A yield of 3.8 Gm. (83% of theoretical) of 2-diethylaminoethyl 2,2,3-triphenylcyclopropanecarboxylate hydrochloride, white crystals, m. p. 134.5 to 135.2°, was obtained.

Anal.—Calcd. for $C_{28}H_{32}ClNO_2$: Cl, 7.88; N, 3.11. Found: Cl, 7.99, 7.88; N, 2.93, 2.93.

2-Diethylaminoethyl 2,2-Diphenylcyclopropanecarboxylate Hydrochloride.—By means of the procedure described for the preparation of the 2,2,3-triphenylcyclopropanecarboxylate, an 81% yield of 2-diethylaminoethyl 2,2-diphenylcyclopropanecarboxylate hydrochloride, white crystals, m. p. 147.5 to 148.2°, was obtained.

Anal.—Calcd. for $C_{22}H_{28}ClNO_2$: Cl, 9.48; N, 3.74. Found: Cl, 9.62, 9.54; N, 3.75, 3.76.

2-Diethylaminoethyl Dibenzonocaradienecarboxylate Hydrochloride.—By means of the procedure described for the preparation of the 2,2,3-triphenylcyclopropanecarboxylate, a 70% yield of 2-diethylaminoethyl dibenzonorcaradienecarboxylate hydrochloride, white crystals, m. p. 192–193°, was obtained.

Anal.—Calcd. for $C_{25}H_{28}ClNO_2$: Cl, 9.54; N, 3.77. Found: Cl, 9.63, 9.50; N, 3.75, 3.75.

2-Diethylaminoethyl 2,3-Diphenylcyclopropanecarboxylate Hydrochloride.—By means of the procedure described for the preparation of the 2,2,3-triphenylcyclopropanecarboxylate, a 52% yield of 2-diethylaminoethyl 2,3-diphenylcyclopropanecarboxylate hydrochloride, white crystals, m. p. 190.6 to 191.4°, reported m. p., 185–188° (3), was obtained.

Anal.—Calcd. for $C_{22}H_{28}ClNO_2$: Cl, 9.48; N, 3.74. Found: Cl, 9.60, 9.35; N, 3.75, 3.75.

SUMMARY

1. A series of 2-diethylaminoethyl cyclopropanecarboxylates, in which the acid moiety is substituted by two or three aryl groups or is associated with a condensed polynuclear ring system, has been prepared for testing as spasmolytic agents.

2. Details are given for the preparation of 2,3-diphenylcyclopropanecarboxylic acid by reaction of ethyl diazoacetate with stilbene followed by saponification of ethyl 2,3-diphenylcyclopropanecarboxylate which is formed in the reaction.

3. The effect of copper powder and the use of cetane as an inert solvent in the reaction of ethyl diazoacetate with phenanthrene is described.

4. The preparation of the following basic ester hydrochlorides by reaction of the sodium salts of the acids with 2-chloroethyldiethylamine is described: 2-diethylaminoethyl 2,2-diphenylcyclopropanecarboxylate hydrochloride; 2-diethylaminoethyl 2,3-diphenylcyclopropanecarboxylate hydrochloride; 2-diethylaminoethyl 2,2,-3-triphenylcyclopropanecarboxylate hydrochloride; and 2-diethylaminoethyl dibenzonorcaradienecarboxylate hydrochloride.

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Titration of Barbiturates and Sulfa Drugs in Nonaqueous Solution*,†

By VINCENT VESPE‡ and JAMES S. FRITZ§

Barbiturates are weak acids and give poor end points when titrated in alcohol or water. Titration in dimethylformamide (DMF) with sodium methoxide in benzene-methanol, however, gives very sharp visual end points. Tablets can be assayed for barbiturate or sulfa content by direct titration of a powdered sample. This method is convenient and much more rapid than the official method for barbiturates. For sulfa drugs, the acidimetric method is faster and more accurate than the nitrite method usually employed.

CURRENTLY there is considerable interest in acid-base titrations in nonaqueous solvents. By proper choice of solvents, many substances may be determined which are too weakly basic or acidic to be titrated in water or alcohol; yet the speed, convenience, and accuracy commonly associated with titrations in aqueous solution are retained.

Moss, Elliott, and Hall (1) showed that phenol and other weakly acidic compounds behave as fairly strong acids when titrated in a basic solvent such as ethylenediamine. Excellent end points were obtained potentiometrically, but satisfactory visual indicators were not found. Fritz and Lisicki (2) showed that weakly acidic compounds could be accurately titrated using thymol blue indicator and benzene-methanol, butylamine, or dimethylformamide (DMF) as the solvent. A recent paper by Fritz and Keen (3) reported the titration of sulfonamides as acids in DMF or butylamine.

It is now proposed to determine barbiturates and sulfa drugs in various pharmaceutical preparations by acidimetric titration in nonaqueous solution. The sample is simply dissolved in DMF and titrated with 0.1 *N* sodium methoxide, using thymol blue indicator. The end point is marked by a sudden vivid change from yellow to blue.

EXPERIMENTAL

Reagents.—(*a*) Benzene, purified grade.

(*b*) Benzoic acid, primary standard grade.

(*c*) Dimethylformamide (DMF), technical (du Pont).

(*d*) Methanol, absolute as purchased commercially.

(*e*) Sodium methoxide, 0.1 to 0.2 *N*. Dissolve about 3 Gm. of freshly cut sodium in 50 ml. of methanol, protecting the solution from CO_2 while the sodium is reacting. Add 100 ml. of methanol and 750 ml. of benzene, and store the reagent in borosilicate glassware. The solution should be essentially colorless.

(*f*) Thymol blue solution. Dissolve 0.3 Gm. of thymol blue in 100 ml. of methanol.

Procedure.—The size of the sample should be such that about 0.3 to 0.8 milliequivalent of acidic substance is present. The sample is dissolved in 20–30 ml. of DMF, two drops of thymol blue added and titrated with 0.1 *N* sodium methoxide to a clear blue color. A microburette (10-ml.) which can be read accurately to 0.01 ml. is used instead of the usual 50-ml. burette. For best results, the solution should be protected from carbon dioxide in the atmosphere during the titration. This is conveniently done by carrying out the titration in a small flask or beaker covered by cardboard with a hole to admit the burette tip. Use of a magnetic stirrer adds to the convenience of the titration. The titrant is standardized by titration against benzoic acid.

DMF always contains some acidic impurities for which a correction must be made. In all the titrations reported, a blank of 0.02 ml. of 0.1 *N* sodium methoxide was required for approximately 25 ml. of DMF.

Barbiturates.—Table I gives quantitative data for the titration of 12 pure barbiturates. All of the barbiturates are readily soluble in DMF and give very sharp visual end points.

The phenobarbital content of several pharmaceuticals was determined by the official method. The residue was weighed and then dissolved in DMF and titrated with sodium methoxide. Table II shows that the gravimetric and titrimetric methods are in excellent agreement. The titrimetric method obviates the use of a tared flask and has the further advantage that nonacidic substances would not be determined if extracted along with the barbiturate.

Finally, a powdered tablet sample was dissolved in DMF and titrated directly. Results obtained show that the extraction procedure is unnecessary and that the phenobarbital may be titrated accurately directly on the powdered sample.

Sulfonamides.—Acidimetric titration of sulfa drugs in DMF [first reported by Fritz and Keen (3)] is applicable for the assay of tablets. Results obtained agree with the nitrite method, but acidi-

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‡ The J. F. Hartz Co., Ferndale, Mich.

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TABLE I.—TITRATION OF PURE BARBITURATES (100-MG. SAMPLES TAKEN IN EACH CASE)

Compound	Ml. Base	N of Base	% Recovery	Av. % Recovery
Amobarbital	3.44	0.1285	99.8	
	3.44	0.1285	99.8	
	3.45	0.1285	100.2	
	3.82	0.1158	100.0	
	3.83	0.1158	100.2	100.0
Barbital	4.26	0.1285	100.5	
	4.24	0.1285	100.2	
	4.69	0.1158	100.2	
	4.69	0.1158	100.2	
	4.67	0.1158	99.6	100.1
Delvinal	3.81	0.1170	99.8	
	3.81	0.1170	99.8	
	3.87	0.1158	100.2	
	3.88	0.1158	100.6	
	3.86	0.1158	100.1	100.1
Pentobarbital	3.48	0.1258	100.5	
	3.45	0.1258	99.7	
	3.46	0.1258	100.0	
	3.82	0.1158	99.5	
	3.82	0.1158	99.5	99.8
Pentothal	3.28	0.1258	99.8	
	3.28	0.1258	99.8	
	3.30	0.1258	100.2	
	3.56	0.1158	99.7	
	3.57	0.1158	99.9	99.9
Phenobarbital	3.54	0.1225	100.5	
	3.54	0.1225	100.5	
	3.42	0.1258	99.8	
	3.44	0.1258	100.3	
	3.70	0.1169	100.5	100.3
Seconal	3.30	0.1285	100.5	
	3.26	0.1285	99.3	
	3.26	0.1285	99.3	
	3.66	0.1158	100.3	
	3.65	0.1158	100.2	99.9
Surital	3.12	0.1258	99.6	
	3.14	0.1258	100.3	
	3.14	0.1258	100.3	
	3.41	0.1158	100.2	
	3.40	0.1158	100.0	100.1
2-Ethylbutyl-ethylbarbituric acid	3.31	0.1258	99.9	
	3.30	0.1258	99.7	
	3.61	0.1258	100.2	
	3.60	0.1158	100.1	
	3.60	0.1158	100.1	100.0
2-Methylbutyl-ethylbarbituric acid	3.51	0.1258	99.8	
	3.51	0.1258	99.8	
	3.83	0.1158	100.2	
	3.82	0.1158	99.9	
	3.82	0.1158	99.9	99.9
1-Methylpropyl-allylbarbituric acid	3.54	0.1258	99.7	
	3.56	0.1258	100.2	
	3.85	0.1158	99.8	
	3.87	0.1158	100.3	
	3.86	0.1158	100.2	100.0

metric titration is more convenient and the precision is much greater. Data are given in Table III.

Sulfasuxidine and sulfathalidine, which lack the primary amino group necessary for titration with nitrite, can be determined by the acidimetric method. Sulfaguanidine is the only common

TABLE II.—DETERMINATION OF PHENOBARBITAL IN PHARMACEUTICALS

Sample	Official Method, Mg.	Titration of Residue, Mg.	Titration of Powder Sample
Tablet 1	98.0	98.0	98.0
Tablet 2	98.2	98.0	98.2
Tablet 3	97.5	97.7	97.7
Tablet 4	98.0	98.0	98.0
Tablet 5	97.7	97.5	97.8
Tablet 6	98.0	98.0	98.0
Tablet 7	97.7	97.6	97.8
Tablet 8	97.5	97.5	97.7
Elixir 1	104.0	104.0	...
Elixir 1	105.0	105.0	...
Elixir 1	105.0	104.0	...
Elixir 1	104.5	104.0	...
Elixir 1	105.0	105.0	...
	(Av. 104.7)	(Av. 104.4)	...
Elixir 2	99.8	99.8	...
Elixir 2	100.1	100.0	...
Elixir 2	100.1	100.0	...
Elixir 2	99.7	99.8	...
	100.0	99.8	...
	(Av. 99.9)	(Av. 99.9)	...

TABLE III.—TITRATION OF SULFONAMIDES IN TABLETS

Sample	Nitrite Method, Mg.	Acidimetric Method, Mg.
Sulfadiazine	496	502
	505	501
	502	499
	495	500
	497	498
	(Av. 499)	(Av. 500)
Sulfathiazole-sulfadiazine	508	502
	502	501
	495	500
	502	501
	504	501
	(Av. 502)	(Av. 501)

member of the sulfa group which cannot be titrated as an acid.

Interferences.—Most common tablet excipients are not acidic, hence do not interfere with the method. One-gram samples of potato starch, powdered sugar, and magnesium stearate gave negligible blanks. Magnesium trisilicate and kaolin (0.5-Gm. samples) required approximately 0.2 ml. of 0.1 *N* sodium methoxide.

Caffeine, which frequently occurs with barbiturates in pharmaceuticals, does not interfere. Theobromine is an acid in DMF, but it should be possible to avoid by extracting the barbiturate with chloroform or some other solvent in which theobromine is slightly soluble.

Water interferes, due to partial hydrolysis of DMF, although trace amounts can be tolerated. Enols, imides, and other acidic substances also interfere.

Scope.—A great many acidic substances may be accurately titrated as acids in DMF and other nonaqueous solvents. Work is in progress which is expected to show that theophylline, theobromine, hydrantoins, thiouracils, and other well-known pharmaceuticals can be titrated in this manner.

SUMMARY

1. Barbiturates can be accurately titrated as acids in nonaqueous solution using a visual end point.

2. Titration of phenobarbital extracted from tablets gives results in agreement with the official gravimetric method and obviates the use of tared flasks.

3. Barbiturates and sulfa drugs can also be accurately determined by direct titration of powdered tablet samples.

4. Interferences, limitations, and possible further applications of the method are stated.

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Investigation of the Toxic Principles of *Hippomane mancinella*, L. I. Historical Review*

By W. M. LAUTER,† LAURETTA E. FOX,‡ and WILLIAM T. ARIAIL§

***Hippomane mancinella* L., family *Euphorbiaceae*, which grows in southern Florida and other parts of tropical America, is frequently mentioned in the chronicles of the early explorers of the Americas. The Caribs used the sap of the tree as an arrow poison. The fruits, which resemble crab apples, were frequently consumed, often with fatal results. Contact with the sap of the tree or the leaves is reported as causing severe inflammation of the skin or sometimes temporary blindness. Several native plants are used as antidotes.**

THE plant *Hippomane mancinella* L., family *Euphorbiaceae*, grows on the seashores of many parts of tropical America, where it forms dense thickets along both the Atlantic and Pacific beaches, extending into Mexico, the West Indies, Venezuela, the Guianas, and southern Florida (1), where it grows as far north as Palm Beach and Fort Myers. West and Arnold (2) consider it the most toxic tree on the North American continent.

The tree is from 10 to 20 feet high, with trunks measuring from 5 to 6 inches in diameter. The crown is rounded, composed of long, drooping branches, giving it the appearance of a fruit tree. The dark green leaves are 2 to 4 inches long, and arranged alternately. The bark is dark brown, usually roughened with small thick plates. The fruits are spherical or elongated, 1 to 1½ inches in diameter, and colored light yellow-green with bright red cheeks. They mature in the fall. Because they resemble crab apples they have been mistaken for these edible fruits and their consumption resulted in death.

The wood is yellowish brown variegated with brown and black, of medium weight and hardness, durable, and of a fine texture resembling walnut. Since the wood takes a good polish it is excellent for cabinet work. Working with the trees and wood is hazardous, and great care must be taken. Woodcutters build a fire around the trunks and char the bark before cutting down the trees (3).

History of Toxic Manifestations

Few specimens of *Hippomane mancinella* L. are found near populated areas since it was destroyed by the early settlers. However, the literature abounds in descriptions of its noxious qualities. The following excerpts from the historical literature dealing with *Hippomane mancinella* L. [manchineel (English), manzanillo (Spanish), mancinillier (French)] give a picture of the opinions of the plant held by the early explorers in the Americas.

One of the earliest references is in the writings of Peter Martyr, the court geographer of Queen Isabella, patroness of Columbus, who wrote in *De Orbe Novo* in 1509 (4):

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"Upon the same banks there grows a tree whose fruits are sure poison, and yet they are sweet. When the fruits fall into the water they are eaten by fish, people who afterwards eat those fish are attacked by divers strange maladies. The Friar Tomas Ortis declared that he has tasted, but not eaten, these fruits; their taste is bitter-sweet. He was afterwards slightly indisposed. The antidote to this poison is to swallow oil. Even the shade of this fruit-tree affects the head and hurts the eyes. Dogs, cats and other animals which eat of it die."

Manchineel, as well as other plants, was widely used by the Carib Indians of South America as an arrow poison, and again Martyr (5) writes:

"They like to use bows and poisoned arrows. They poison their arrows with the stings of scorpions, the heads of certain ants, poisons which they manufacture and those little plums[1] which I have mentioned, as well as the juice they distill from certain trees in which they dip their arrows."

According to Standley (1), "Contributions from the United States National Herbarium," Vol. 23, Part 3, Ovideo cites one case in which a man ate five or six fruits and recovered, while Madaus (6) records the recovery of one man after consuming 24 fruits.

Ovideo, according to Standley (1), writes further:

"In these regions are many *manzanillos*, with which and other poisonous mixtures the Indians are accustomed to make that diabolic poison for their arrows. These trees are usually low or spreading and some are more than six yards high; they have large tops full of leaves which resemble those of the pear. The trees are loaded with a fruit like apples, of good odor, as large as pears but round, or sometimes elongate, variegated with red, which gives them a pleasing appearance; but both they and the trees are poisonous in their effects. In Hispaniola the Indians do not use the plant, but there is no man who sees the fruit, if he does not know it, who does not wish to feed upon it, for its appearance and odor are inviting. It has been proved many times that if men carelessly lie down to sleep under the trees, when they rise after a short nap there is a great pain in the head and swelling of the eyes and cheeks. And if by chance the dew from the tree falls on the face, it is like fire, blistering and burning the skin wherever it touches; and if it falls in the eye it blinds or burns them, and the sight is endangered. If the wood is burned no one can endure it long, for it causes much heaviness, and such headaches that all stand away from it, be they man or any other animal."

Richard Ligon (7), a physician of the island of Barbadoes, wrote in 1673:

"The Poysoned tree, though I cannot comment on her vertues, yet for her beauties I can. She is almost as large every way as the locust, but not of that manner of growing, and so like, as not to be known asunder. The people that have lived long there, say, 'tis not wholsom to be under the shade of this tree. The fellers, as they cut them down, are very careful of their eyes; and those that have Cipers, put it over their faces; for if any sap fly in their eyes, they become blind for a month. A negro had two horses to walk, which were left with him by two Gentlemen; and the horses beginning to fight, the negro was afraid, and let them go; and they running into the woods together, struck at one another, and their heels hitting some young plants of this kind, struck the poysonous juice into one anothers eyes, and so their blindness parted the fray, and they were both led home stone blind, and continued so a month, all the hair and skin pilling off their faces. Yet, of this timber we make all, or the most part, of the pots we cure our sugar in; for being sawed, and the boards dryed in the sun the poyson vapours out.

"And as this tree's poyson in her sap, so the Mantionell's is in her fruit, which they count as high a poyson, as that of the Cassavie. The fruit is like an apple John, and 'tis said to be one of those poysons, wherewith the Indian Caniballs invenome their arrows."

One of the more prominent historical figures to suffer from poisoning from manchineel was Admiral Horatio Nelson, the hero of Trafalgar. His contemporary biographer, Robert Southey, states that while Admiral Nelson was in command of a British expedition into Spanish Nicaragua, he was poisoned by hostile Indians who had saturated the water in a spring with manchineel leaves. The quotation (8) is as follows:

". . . drinking at a spring in which some boughs of the manchineel had been thrown, the effects were so severe, as, in the opinion of some of his friends, to inflict a lasting injury upon his constitution."

McIntyre (9) in his book, "Curare, its History, Nature, and Clinical Use," quotes from Sir John Hawkins in his "Voyage to Guiana" as follows:

"Their poison is of such force, that a man being stricken therewith, dieth within foure and twentie howres, . . . and in my judgment it is like there can bee no stronger poyson as they make it, vsing thereunto apples which are very fair, and

[1] Mancinillier.

of red colour, but are a strong poyson, with which together with venemous Bats, Vipers, Adders, and other serpents, they make a medley and therewith anoit the same."

According to Standley (1) goats are said to eat the fruits greedily without injurious results.

James C. White (10) in his "*Dermatitis Venenata*," has the following quotation from Kingsley's "At Last":

"We learnt to distinguish the poisonous manchineel, and were thankful in serious earnest that we had happily plucked none the night before, when we were snatching at every new leaf; for its milky juice by mere dropping on the skin burns like the poisoned tunic of Nessus, and will even, when the head is injured by it, cause blindness and death."

The whole tree abounds in a white, caustic, venomous juice which has been used in medicine. In Jamaica it has been used in the treatment of dropsy and venereal disease, and the seeds and bark have been employed as a vermifuge (1), in spite of the fact that their use is extremely dangerous. "The United States Dispensatory," 24th ed. (11), states that in doses of two drops it is a powerful and a very certain cathartic and is also diuretic. According to Grieve and Leyel (12) it is widely used in Cuba for tetanus.

Hence the historical data appear to indicate that there are several actions on man and animal:

1. Eating the fruit may cause death. One person recovered from twenty-four fruits; usually five or six can be consumed without causing death from acute poisoning.

2. Contact with the sap of the tree or the leaves appears to cause severe inflammation of the skin and temporary blindness.

Antidotes

It is interesting to note that references in the literature indicate that the natives of the tropical Americas consider several plants as antidotes against the toxic actions of *Hippomane mancinella* L. *Adenoropium gossypiifolium* (L.) Pohl., referred to in the earlier literature as *Jatropha gossypiifolium* L., has been used as an antidote for manchineel in Mexico (13). Nearly all species of *Jatropha* yield fixed oils which serve as purgatives. *Tabeuia pentaphylla* (L.) Hemsl., the blue trumpet flower, called by Madaus (6) *Bignonia leucoxylon* L. and by Combs according to Pammel (14) *Tecoma leucoxylon* Mart., has also been used as an antidote, as well as *Bignonia unguiscati* L. (15). Pammel (14) states that Combs also reported in his paper on Cuban Medical Plants that the uncooked rhizome of *Maranta arundinaceae* L. is sometimes used for the same purpose.

Although the earlier literature states that the "blue trumpet flower," *Tabeuia pentaphylla* (L.) Hemsl. grows in close proximity to the manchineel, it could not be located by the senior author in the stands of machineel in the Everglades of southern Florida.

From the above it appears that *Hippomane mancinella* L. possesses an unusual number of interesting, highly toxic constituents, which are worthy of chemical and pharmacological study.

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The Synthesis of Some New Esters of *p*-Carboxybenzamidine*,†

By ALFRED W. CHOW and OLE GISVOLD‡

Because it had previously been shown that some of the simple *n*-alkyl-*p*-carboxybenzamidines possessed significant local anesthetic activity, several new esters were prepared in order to test their activities. Representative examples of halogenated alcohols, aromatic alcohols, and cyclohexanol were chosen for the alcohol portion of the molecule. Several of these new esters were more active than both cocaine when instilled in guinea-pig eyes and procaine when administered intracutaneously in guinea-pig skin. Toxicities were favorable; however, they produced an undesirable pruritis when injected in the forearm of man.

IN A PREVIOUS publication a number of *n*-alkyl esters of *p*-carboxybenzamidine were prepared (1) and a subsequent screening of their local anesthetic activities showed that the esters prepared from *n*-amyl and *n*-hexyl alcohol and *p*-carboxybenzamidine had definite anesthetic activity. As one phase of a continuation of this field of research, it was deemed advisable to prepare esters other than the *n*-alkyl series to test them for their possible anesthetic activity. A miscellaneous group of esters has been prepared and some of these compounds exhibited marked topical and infiltration anesthesia.

EXPERIMENTAL

The esters were prepared essentially by the procedure described by DiGangi and Gisvold with some modifications. *p*-Cyanobenzoic acid was prepared by the usual procedure (2) with some modifications that will be the subject of a future publication.

***p*-Cyanobenzoyl Chloride.**—This intermediate was prepared as previously described (1) with the exception that the crude acid chloride was recrystallized from absolute ether.

***p*-Cyanobenzoic Acid Esters.**—These esters were made by direct condensation of the acyl chloride and alcohol or by using pyridine as a catalyst. As an illustration of the former procedure, 16.5 Gm. (0.1 *M*) of *p*-cyanobenzoyl chloride and 14.4 Gm. (0.1 *M*) of 2,3-dichloropropanol were heated on a steam bath for three hours in a 500-cc. round-bottom flask carrying a condenser and a calcium chloride drying tube. After standing overnight at room temperature, the solid mass which had formed was crushed, digested with 5% sodium carbonate solution, filtered, and washed with water. The crude ester thus obtained was recrystallized from alcohol after decolorizing with charcoal.

As an illustration of the latter procedure, 8.5 Gm. (0.05 *M*) of *p*-cyanobenzoyl chloride was dissolved in 20 cc. of benzene to which 5 cc. of *p*-chlorophenol in 5 cc. of dry pyridine was added. The mixture was refluxed on a steam bath for one hour. Upon cooling, the solid mass which formed was filtered off, washed with 5% sodium carbonate solution, then with water, and finally dried. The crude product was recrystallized from benzene and finally from alcohol.

***p*-Carbethoxyimidobenzoic Acid Ester Hydrochlorides.**—These were prepared by the method of Pinner (1, 3); however, in all but two cases dry dioxane gave much better results than dry ether. In those cases where the iminoether hydrochloride did not separate after two to seven days in the ice chest, light petroleum ether was subsequently added. This caused the separation of an oil which, when washed several times with light petroleum ether and finally with anhydrous ether, solidified in a few hours. The above variations can be illustrated by the following experiment: 16.8 Gm. (0.08 *M*) of β-chloroethyl *p*-cyanobenzoate was partially dissolved in 150 cc. of dioxane. Eight cubic centimeters of absolute ethyl alcohol was then added and the reaction mixture was saturated with dry hydrogen chloride gas at 0–5° over a period of an hour in an Erlenmeyer flask equipped with a calcium chloride tube and a gas inlet tube. During this period the cyanoester gradually passed into solution. The flask was stoppered and left in the ice chest for five days but no separation occurred. Light petroleum ether was added until the solution was turbid and the flask returned to the ice chest. In a few hours a white crystalline mass formed which was filtered, washed with anhydrous ether, and dried. A second crop was obtained by adding more light petroleum ether to the mother liquor.

***p*-Carboxybenzamidine Esters.**—The iminoether hydrochlorides were converted to the corresponding amidine hydrochlorides either by warming the iminoether hydrochlorides with a slight excess of 28% ammonium hydroxide (1, 3) in 95% alcohol at 60–80° for two to three hours, or by warming the iminoether free base in 95% alcohol with the theoretical amount of ammonium chloride dissolved in a trace of water (1, 4, 5). Although the above procedures gave the desired *p*-carboxybenzamidine esters, success was not encountered in all cases. For example, even though diethylaminoethyl *p*-carbethoxyimidobenzoate could be prepared, con-

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† Abstracted from the thesis submitted to the Graduate Faculty of the University of Minnesota by Alfred W. Chow in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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TABLE I.—ESTERS OF *p*-CYANOBENZOIC ACID

No.	Ester Residue	Method	Yield, %	M. P., °C.	Analysis, Calcd.	% C and H Found
1	2-Chloroethyl	A[a]	65.8	87–90	C, ...	...
					H, ...	...
2	2-Bromoethyl	A	74.8	89–90	C, 47.26	C, 46.99
					H, 3.17	H, 3.47
3	2,3-Dichloropropyl	A	62.0	93–95	C, 51.19	C, 51.64
					H, 3.57	H, 3.63
4	2,3-Dibromopropyl	A	51.9	78–79	C, 38.07	C, 38.03
					H, 2.62	H, 2.80
5	2,2,2-Trichloroethyl	B	63.5	91–93	C, 43.12	C, 43.72
					H, 2.17	H, 2.37
6	Benzyl	A	85.9	56–57.5	C, 75.92	C, 76.14
					H, 4.68	H, 4.73
7	Cyclohexyl	A	71.3	62–63	C, 73.33	C, 73.14
					H, 6.59	H, 6.79
8	Dimethylaminoethyl	A	98.4	197–198	C, ...	...
					H, ...	...
9	Diethylaminoethyl	A	88.2	157.5–159.5	C, ...	...
					H, ...	...
10	*p*-Chlorophenyl	B[b]	45.0	142–142.5	C, 65.27	C, 65.11
					H, 3.13	H, 3.43
11	Furfuryl	B	91.9	72–73	C, 68.69	C, 68.45
					H, 3.99	H, 4.17
12	Tetrahydrofurfuryl	B	64.6	40–42	C, ...	...
					H, ...	...

[a] Method A: Direct interaction of acyl chloride and alcohol.
[b] Method B: Using pyridine as a catalyst.

TABLE II.—ESTERS OF *p*-CARBETHOXYIMIDOBENZOIC ACID HYDROCHLORIDE[a]

No.	Ester Residue	Anhydrous Solvent	Method	Yield, %	M. P., °C.	Analysis Calcd.	Analysis Found
1	2-Chloroethyl	Dioxane	I[c]	68.7	163–164	...	...
2	2-Bromoethyl	Dioxane	I	86.3	169–170	...	...
3	2,3-Dichloropropyl	Dioxane	II[d]	91.1	119–120	...	...
4	2,3-Dibromopropyl	Dioxane	II	82.4	112–113	...	...
5	2,2,2-Trichloroethyl[b]	Dioxane	I	74.9	212–214	...	...
6	Benzyl	Ether	III[e]	87.7	114–115	...	...
7	Cyclohexyl	Ether	III	66.8	108–109	...	...
8	Dimethylaminoethyl	Dioxane nitrobenzene	I	77.0	97–101	...	...
9	Diethylaminoethyl	Dioxane	II	93.1	128–129	C, 52.62 H, 7.18	C, 52.35 H, 7.09

[a] Iminoether hydrochloride not obtained: furfuryl. Iminoether hydrochloride obtained in very low yields: tetrahydrofurfuryl and *p*-chlorophenyl.
[b] Using ether as a solvent, compounds 1–5 could not be obtained in good yield.
[c] Method I: Dry dioxane as solvent, product separated as a solid.
[d] Method II: Dry dioxane as solvent, product separated out as an oil.
[e] Method III: Anhydrous ether as solvent, product separated out as a solid.

TABLE III.—ESTERS OF *p*-CARBOXYBENZAMIDINE HYDROCHLORIDE[a]

No.	Ester Residue	Method	Yield, %	M. P., °C.	Analysis, Calcd.	C and H Found
1	2-Chloroethyl[c]	1[d]	64.0	199–201	C, 45.62	C, 45.33
					H, 4.60	H, 4.63
2	2-Bromoethyl[b]	1	43.8	200–201[f]	C, 39.04	C, 39.65
					H, 3.93	H, 4.23
3	2,3-Dichloropropyl	1	79.1	168–169	C, 42.79	C, 42.39
					H, 4.25	H, 4.35
4	2,3-Dibromopropyl	1	75.4	159–160	C, 32.97	C, 33.16
					H, 3.25	H, 3.15
5	Benzyl[c]	1	88.0	181–182	C, 70.19	C, 70.17
					H, 5.14	H, 5.35
6	Cyclohexyl	1	90.0	202–203	C, 59.48	C, 58.95
					H, 6.79	H, 6.73
7	2,2,2-Trichloroethyl	2[e]	65.3	204–206	C, 36.07	C, 36.42
					H, 3.33	H, 3.34

[a] Compounds not obtained: dimethylaminoethyl, diethylaminoethyl.
[b] The identity of this compound is questionable.
[c] Analyzed as the ester benzoate.
[d] Method 1: Using 28% ammonium hydroxide.
[e] Method 2: Using ammonium chloride.
[f] The melting point of this compound was not sharp.

TABLE IV.—ANESTHETIC ACTIVITY OF SOME ESTERS OF *p*-CARBOXYBENZAMIDINE

Compound R=	Concn. of Solution, %	Duration of Anesthesia, Guinea Pigs— Eyes, Min.	Skin, Min.	Irritation, Rabbit Skin	LD50, Mg./Kg., Mice— Intravenous	Subcutaneous
n-Propyl	1	0	10	Severe	65	550
n-Butyl	1	0	45	Severe	70	350
	0.1		28	Moderate	...	...
n-Amyl	1	26	28	Severe	105	450
n-Hexyl	1	0	145	Severe	150	550
	0.1		20	Moderate	...	...
2-Chloroethyl[a]	1	0	155	Negligible	84.8	...
	0.5		90	Negligible	...	...
	0.25		0	...	...	...
2,2,2-Trichloroethyl	1	0	0	...	...	...
2,3-Dichloropropyl	1	0	25	...	...	...
2,3-Dibromopropyl	1	0	0	...	...	...
Cyclohexyl	1	130	360	Moderate to severe	112	...
	0.25	11	120	...	...	...
	0.1		20	Negligible		
Benzyl[b]	1	108	180	Moderate	58.7	...
	0.5	59				
	0.25	17	126	...	...	...
	0.1		85	...	...	...
	0.05		0	...	...	...

[a] Incomplete anesthesia, intradermally in man, plus initial stinging and widespread erythema for one and one-half hours.
[b] Man, intradermal injection of 0.1-cc. doses of a 0.25% solution gave a slight stinging on injection followed by intense pruritis over entire volar surface of forearm. Complete anesthesia was produced for one and one-third hours in one of two subjects.

version to the corresponding amidine by the above methods was unsuccessful. Ethyl *p*-amidinobenzoate was the only product that could be obtained and it was assumed that ester interchange had taken place along with the conversion of the iminoether group to the amidine group.

Several attempts to prepare the amidines directly from the corresponding *p*-cyanobenzoates by fusion with ammonium thiocyanate were unsuccessful (6). *p*-Carbamidobenzamidine was the only product that was identified as a result of the above reaction and it was assumed that ammonolysis occurred together with conversion of the cyano group to the amidine.

Tables I–III summarize the results obtained in the synthesis of the intermediates and final products, i.e., the esters of *p*-carboxybenzamidine.

Anesthetic Activity.—Through the courtesy of K. K. Chen of Eli Lilly and Company, the esters prepared and reported in this paper, together with those previously reported (1), were tested for their anesthetic activity by C. L. Rose. The results of these tests are tabulated as shown in Table IV.

SUMMARY

A practical procedure for the preparation of *p*-cyanobenzoic acid in 50 to 60 per cent yield has been described.

Ten new esters of *p*-cyanobenzoic acid were prepared from *p*-cyanobenzoyl chloride and the appropriate alcohols.

Nine new esters of *p*-carbethoxyimidobenzoate hydrochloride were prepared by Pinner's method.

Six new amidines (*p*-amidinobenzoates) were prepared by Pinner's method and modifications thereof.

The preparation of *p*-(dialkylaminoalkylcarboxy) benzamidines was not successful.

The anesthetic activity of the *p*-carboxybenzamidine esters has been reported, together with several others that had been previously prepared.

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A Modification of the Gold Bromide Method for Determining Bromides in Blood and Spinal Fluid*,†

By W. D. PAUL, R. W. KNOUSE, and J. I. ROUTH

A modification of the procedure for the color reaction between gold chlorides and bromides has been devised and is described. This modification requires only half the volume of body fluids necessary for methods previously described, eliminates centrifuging, and is less time-consuming. The optimum concentrations of sodium chloride and of trichloroacetic solutions employed were found to be 0.35 per cent and 5 per cent, respectively.

SINCE Walter (1) first described the color reaction between gold chloride and bromides, his procedure for bromide determination has undergone several modifications. Hauptman (2) adapted the reaction to the colorimeter. Wuth (3) devised a clinical method in which the gold bromide color was compared with tubes containing certain dyes. In both the Hauptman and Wuth procedures, utilizing a trichloroacetic acid precipitation of proteins, there was an apparent loss of bromide. Wuth attributed the loss to the retention of bromide by the protein precipitate, but Friedman (4) was able to obtain quantitative recovery of bromide from plasma. Friedman oxidized the bromide to bromate and determined the latter iodometrically. Katzenelbogen and Czarski (5) demonstrated that complete bromide recovery was possible by the Hauptman or Wuth procedures if the serum was diluted with 0.75 per cent saline instead of water. As the sodium chloride concentration was increased beyond 0.75 per cent, the relative intensity of the gold bromide color decreased. These investigators also tested several organic and inorganic compounds and found that none affected the gold bromide color except sodium chloride.

We have attempted to simplify the method, to shorten the time involved, and to permit the use of smaller samples, without sacrificing accuracy.

EXPERIMENTAL

To show the importance of the concentration of sodium chloride solutions and the composition of the wash solution, an initial and a final modification of the method of Katzenelbogen and Czarski (5) are presented. The initial modification employed the following procedure:

1. Pipette into a 15 x 85 mm. (approx.) test tube: 1 ml. plasma, serum, or spinal fluid; 2 ml. 0.75% sodium chloride solution; and 1 ml. 20% trichloroacetic acid solution.
2. Stir with a glass rod and break up precipitate to minimize occlusion of filtrate.
3. Moisten filter paper (5.5-cm. Whatman No. 30, or equivalent) in funnel (30–35 mm. diameter) with 0.75% sodium chloride solution, and allow to stand two minutes before filtering. Filter into optical cuvettes (11 x 75 mm.) graduated at 4.0 ml. Wash precipitate with enough 0.75% saline to dilute the filtrate to 4.0 ml. Add exactly 0.5 ml. of 0.5% gold chloride. Mix by inversion and measure optical density in spectrophotometer at a wave length of 470 mμ, using a blank composed of the reagents.

This procedure differs from that of Katzenelbogen and Czarski (5) in the following respects:

1. Only half as much plasma, serum, spinal fluid, and reagents is required.
2. Centrifugation is avoided.
3. The whole filtrate from the trichloroacetic acid precipitation is utilized to give a maximal color change for the amount of bromide present in the sample.
4. The method is adapted to the spectrophotometer.

Using this modification, standard bromide curves were made for saline, plasma, and spinal fluid over a range of 0–500 mg. % bromide. The curves, shown in Fig. 1, were prepared from the data presented in Table I. Since plasma and serum values were nearly identical, they were averaged together. It can be seen from Fig. 1 that the curves are not superimposable. It is apparent that the Lambert-Beer law holds only up to a concentration of about 200 mg. %.

The final modification of the method was instituted to obtain curves which were superimposable up to a bromide concentration of 200 mg. %. Instead of using 0.75% saline, as Katzenelbogen and Czarski (5) suggested, various concentrations were tested. It was found that the curve for bromide in plasma or serum was superimposable upon the saline and spinal fluid curve when a 0.35% saline solution was used throughout the procedure and when the protein precipitate was washed with a solution containing 0.35% sodium chloride and 5% trichloroacetic acid. The addition of the trichloroacetic acid to the wash solution prevented the partial redissolving of the protein precipitate during washing. Employing this additional modification of the method, curves superimposable up to a bromide concentration of 200 mg. % were obtained. The curves, appearing in Fig. 2, were plotted from the values in Table II.

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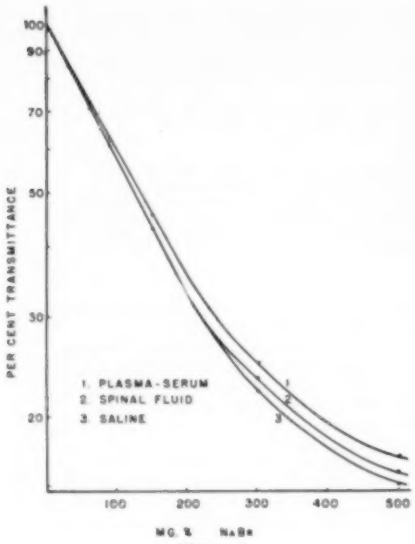

Figure 1

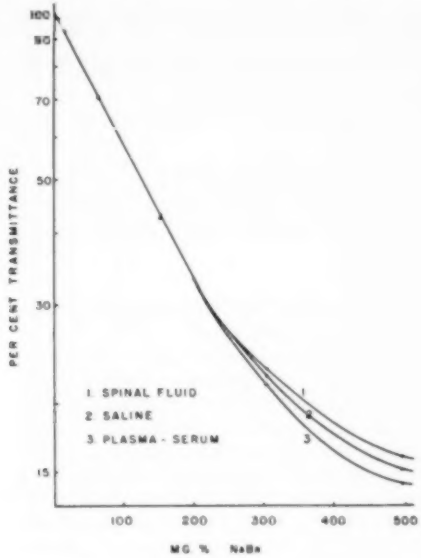

Figure 2

The accuracy of the method was checked by adding known amounts of sodium bromide to plasma, serum, or spinal fluid, and determining recovery by means of the standard curve. Table III presents a comparison of the accuracy of our final modification with the method of Katzenelbogen and Czarski (5).

SUMMARY

1. As compared to previous modifications of the gold bromide color reaction, our modification requires only half as much plasma, serum, spinal fluid, and reagents, avoids centrifuging, uses all of

TABLE I

Concn. Bromide in Mg. %	Saline: % Transmittance[a]	Saline: Max. Deviation in % Transmittance	Spinal Fluid: % Transmittance[a]	Spinal Fluid: Max. Deviation in % Transmittance	Plasma and Serum: % Transmittance[a]	Plasma and Serum: Max. Deviation in % Transmittance
2	98.8	0.4	98.5	0.7	98.7	0.5
4	97.8	1.0	97.5	0.6	97.3	0.6
6	96.6	0.3	96.4	0.8	96.8	0.8
8	95.5	0.4	96.0	1.1	94.5	0.5
15	92.4	0.7	92.3	1.2	91.5	0.9
30	84.0	0.5	84.6	0.9	85.3	0.8
60	70.5	1.0	70.5	1.0	73.0	1.8
90	59.6	1.1	60.8	0.8	62.7	1.2
150	42.9	0.4	43.3	0.5	46.7	0.4
300	21.9	0.7	23.7	0.5	26.5	0.9
500	15.3	0.5	16.1	0.6	17.1	0.8

[a] Average of five determinations.

TABLE II.—COMPARISON OF PER CENT TRANSMITTANCE OF $AuBr_3$ IN VARIOUS SOLUTIONS

NaBr Added, Mg. %	(1) Transmittance[a] in 0.75% Saline, %	(2) Transmittance[a] in Spinal Fluid + 0.75% Saline, %	(3) Transmittance[a] in Plasma or Serum + 0.35% Saline, %	Max. Difference Between 1 and 3, %
4	97.8	97.5	97.3	0.5
8	95.5	96.0	94.7	1.3
30	84.4	84.6	83.2	1.4
60	70.5	70.5	69.1	1.4
90	59.6	60.8	58.9	1.9
150	42.9	43.3	42.1	1.2
300	21.9	23.7	21.0	2.7
500	15.3	16.1	13.9	2.2

[a] Average of five values.

TABLE III[a]

Katzenelbogen and Goldsmith				Our Modification			
Serum		Spinal Fluid		Plasma or Serum		Spinal Fluid	
NaBr Added	NaBr Recovered	NaBr Added	NaBr Recovered	NaBr Added	NaBr Recovered	NaBr Added	NaBr Recovered
50	50.5(+1.0)	10	10.2(+2.0)	10	9.9(−1.0)	10	9.5(−5.0)
100	100(0)	20	20.3(+1.5)	20	19.8(−1.0)	20	19.6(−2.0)
150	149(−0.7)	40	40.0(0)	40	40.1(+0.2)	40	41.0(+2.4)
200	197(−1.3)	60	58.3(−2.8)	60	60.8(+1.3)	60	61.3(+2.2)
250	246(−1.6)	80	80.7(+0.9)	90	87.5(−2.8)	90	90.7(+0.9)
300	297(−1.0)	100	103.5(+3.5)	150	14.7(−2.0)	150	147(−1.9)
				300	288(−4.0)	300	298(−0.7)
				500	494(−1.2)	500	487(−2.5)

[a] Figures are in mg. %; figures in parentheses are differences in per cent.

the filtrate to obtain maximal color intensity, and is generally less time-consuming. It retains the same degree of accuracy as previous methods. The spectrophotometer is used to measure the gold bromide color.

2. By using a 0.35 per cent sodium chloride solution, and a wash solution containing 0.35 per cent sodium chloride and 5 per cent trichloroacetic acid, superimposable standard curves can be prepared for bromide levels in various body fluids.

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Determination of *m*-Aminophenol Content of *p*-Aminosalicylic Acid and Its Sodium Salt*,†

By WILLIAM SEAMAN, J. T. WOODS, W. B. PRESCOTT, and W. H. McCOMAS, Jr.‡

A colorimetric method has been reported for determining *m*-aminophenol (MAP) in *p*-aminosalicylic acid (PAS) and its sodium salt. This method involves diazotization, hydrolysis of the PAS diazo to 2,4-dihydroxybenzoic acid, and coupling of the latter to the MAP diazo. The intensity of the yellow color of the coupling product serves as a measure of the MAP content. The method as reported, which was not satisfactory for medicinal grade PAS containing only small amounts of MAP, was modified to make it suitable for such samples. A study of conditions is reported. The MAP content of a number of samples of PAS ranged from 0.01 to 0.20 per cent, and of sodium PAS from 0.00 to 0.11 per cent. The respective standard deviations were ±0.019 per cent MAP and 0.008 per cent MAP. Brief reference is also made to the color produced by the reaction of MAP and PAS with benzoyl peroxide.

A MEDICINAL of considerable interest in the treatment of tuberculosis, *p*-aminosalicylic acid (PAS) (4-amino-2-hydroxybenzoic acid), may have *m*-aminophenol (MAP) as an impurity. Pesez (1) has published a method for the determination of MAP in PAS in concentrations of from 1 to 10 per cent. This method involves diazotization of the sample, with a subsequent hydrolysis of the diazotized PAS to 2,4-dihydroxybenzoic acid, which couples to the unhydrolyzed diazotized MAP to form a color. The intensity of the color is measured and used to calculate the MAP content of the sample by reference to a curve relating MAP concentration to scale readings on a photoelectric colorimeter. The method appeared to be promising, but left a number of questions unanswered concerning its applicability to the analysis of medicinal grade PAS containing considerably less than 1 per cent MAP rather than MAP in the range of from 1 to 10 per cent. The authors accordingly have carried out investigations for the purpose of applying the method to the lower MAP concentrations. Conditions were determined and fixed for the analysis so that values for MAP which would be precise to within a standard deviation of ±0.019 per cent MAP could be obtained for PAS samples

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‡ Acknowledgment is made to G. A. Allen for assistance with some of the experimental work.

and ±0.008 per cent for sodium PAS samples. A procedure is accordingly recommended for the determination of MAP in PAS and its sodium salt in concentrations below 1 per cent, which is sufficiently rapid and simple for use in routine analysis.

METHOD OF ANALYSIS

The method to be described is essentially that of Pesez (1) but because some modifications in procedure have been introduced, the method is given in detail.

Apparatus.—A Beckman Model DU spectrophotometer or other suitable instrument for the measurement of color.

Reagents.—Sulfuric acid, 1 vol. concd. H_2SO_4 + 9 vol. water; sodium carbonate, 10% Na_2CO_3 (by wt.), clarified, if necessary, by filtration; sodium nitrite, 1.00 Gm. reagent grade $NaNO_2$ made up to 100 ml. with distilled water; sodium hydroxide, approximately normal, reagent grade.

Procedure for PAS.—Pipette into a 100-ml. volumetric flask containing 0.500 Gm. of PAS, 5 ml. of approximately normal NaOH and 5 ml. of distilled water. Dissolve and then dilute to a volume of approximately 80–85 ml. Add by pipette 10.0 ml. of H_2SO_4 (1 + 9) as rapidly as possible, quickly dilute the contents of the flask to the mark with distilled water and mix well. Immediately pipette 5.0 ml. of this solution into a second 100-ml. volumetric flask immersed in an ice bath and containing 50 ml. of distilled water cooled to 0–5°. It is very important to minimize the time during which the sample remains in the sulfuric acid in order to minimize decomposition to MAP. When properly carried out, only two to three minutes should be required for this whole operation. Now pipette in with a Mohr pipette as quickly as possible 2.50 ml. of the sodium nitrite solution. Mix and allow the diazotization to proceed for exactly three minutes (plus or minus five seconds) while the flask remains immersed in the ice bath. Remove the flask from the ice bath and immediately add 25 ml. of 10% sodium carbonate solution. Mix and then place the flask in a water bath at room temperature for about fifteen minutes. Dilute to the mark with distilled water and mix well. Within a period no longer than one hour measure the absorbancy of the yellow solution at a wave length of 440 mμ in a 1.0-cm. cell in a Beckman Model DU spectrophotometer against distilled water as a reference liquid.

Let A = absorbancy.[1] Then

$$\% \text{ MAP} = \frac{A - 0.372}{0.84}$$

where 0.372 is a correction for the decomposition of PAS to MAP and for other possible side reactions which lead to high values for MAP.

The spectral curve of the color produced in the analysis of a sample of PAS is shown in Fig. 1.

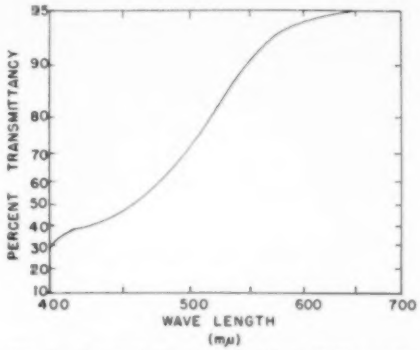

Fig. 1.—Spectral curve of color from PAS sample.

Procedure for Sodium PAS.—Transfer 0.6895 Gm. of the sample of sodium PAS dihydrate into a 100-ml. volumetric flask by means of a few milliliters of distilled water. Pipette into the flask 1.8 ml. of approximately normal sodium hydroxide and dilute to approximately 80–85 ml. From this point, proceed exactly as described previously under "Procedure for PAS," beginning with the addition of 10 ml. of the (1 + 9) sulfuric acid.

Let A = absorbancy. Then

$$\% \text{ MAP} = \frac{A - 0.372}{1.16}$$

DEVELOPMENT AND STUDY OF METHOD

Preparation of Standard ("MAP-Free") Sodium PAS.—It is known that sodium PAS shows a lesser tendency toward decarboxylation with the formation of MAP than does PAS itself (3). A sample of sodium PAS was accordingly purified by recrystallization from two different solvents until a constant absorbancy was obtained for the color produced in the diazotization and coupling method. A good technical grade of sodium PAS dihydrate which had previously been recrystallized from aqueous solution in low yields (sample 1, Table I) was used as a starting material. Sixty grams was dissolved in 2,400 ml. of 3A denatured alcohol and the solution was treated with 5 Gm. of Darco. The solution was then split into two equal parts and each part treated with 3 L. of anhydrous ethyl ether with good stirring. The precipitate was allowed to settle, filtered, washed with four separate portions of 100 ml. each of anhydrous ether (after combining the solids from both original portions) and air-dried. Forty-eight grams of the product was dissolved and again precipitated as described to produce sample 2 of Table I, using proportional quantities of solvents but omitting the addition of Darco. In addition, a third precipitation was carried out on the twice-precipitated material to produce sample 3 (Table I). In order to demonstrate that MAP may readily be removed by this purification process, another portion of sodium PAS which had been recrystallized from water was dissolved in 3A alcohol and sufficient MAP was added to amount to about 0.3% added MAP. Two successive precipitations were made to produce sample 4 of Table I. Samples 2, 3, and 4 gave values for water when analyzed by the two-solution Karl Fischer reagent (4) which corresponded to that of dihydrated sodium PAS. (Sample 1 is also known to be the dihydrate.) From the absorbancy values shown in Table I, it is evident that con-

[1] The spectrophotometric nomenclature used in this paper is that recommended by Mellon (2).

TABLE I.—ABSORBANCY VALUES FOR PURIFIED SODIUM PAS

Sample	Treatment	Absorbancy
1	Recrystallized from aqueous solutions	0.364, 0.383, 0.381, 0.380, 0.371, 0.383, 0.383; av. 0.378
2	Sample 1, pptd. two times from alcohol-ether	0.364, 0.365, 0.364, 0.364, 0.364, 0.365, 0.367; av. 0.365
3	Sample 1, pptd. three times from alcohol-ether	0.375, 0.377, 0.380; av. 0.377
4	Sample 1, pptd. two times after adding 0.3% MAP	0.381, 0.381, 0.381; av. 0.381

stant absorbancy was achieved by this process of purification except for negligible variations. (Even sample 2 differs from the others by an amount equivalent to only about 0.01% MAP). It is realized, of course, that a bare possibility exists that some MAP may still have been present, but that this possibility is a remote one is indicated by the fact that the original sample of sodium PAS had itself been purified by an entirely different process (from aqueous solution) but had furnished, nevertheless, absorbancy values in agreement with those obtained on the samples purified additionally by alcohol and ether.

Establishment of Standard Equation.—The slope of the standard curve which correlates absorbancy and MAP content was established by measuring the increase in the absorbancy which resulted from the addition of known amounts of MAP to a number of samples of medicinal grade PAS in the following manner:

Four portions of 0.500 Gm. each of one sample were dissolved in dilute NaOH as described in the proposed method. Additions of 1.00, 2.00, and 3.00 mg. of MAP, respectively (equivalent to 0.20, 0.40, and 0.60%, respectively, of added MAP), were made to these solutions by means of a volumetric solution of known MAP content. These solutions were then acidified, diluted to volume, and aliquots diazotized and coupled exactly as described in the proposed procedure. The absorbancies of the colored solutions thus obtained were plotted on linear graph paper against the percentage of MAP added. The slope of the best straight line through the four points (including that corresponding to no addition of MAP) was determined. Similar plots were prepared on each of two different days for a similar series of additions of MAP made to each of 10 samples of PAS. (For each of two samples, a series was prepared on one day only.) The average slope determined from these 22 individual curves corresponded to 0.84 absorbancy unit for 1% of MAP added. The precision of this standardization value is expressed by a standard deviation[3] of ±0.067 absorbancy unit. The mean 0.84 has a standard error of ±0.014. The standardization values found ranged between 0.69 and 0.95 for 1% of MAP added.

The straight line which correlates absorbancy and MAP content may be expressed by the equation:

$$\% \text{ MAP} = \frac{\text{Absorbancy} - 0.372}{0.84}$$

In this expression the term 0.372 is the correction to be applied to the measured absorbancy to compensate for the color produced other than by the diazotization and coupling of the MAP initially present in the sample. It should be noted that this correction is applicable only when the conditions prescribed above for the method are adhered to closely. The term 0.84 is the slope of the standard curve expressed as the fraction of an absorbancy unit for 1% MAP when a sample weight of 0.500 Gm. of PAS is taken. This term becomes 1.16 when a sample weight of 0.6895 Gm. is taken as in the procedure for sodium PAS.

The assumption is made that the correction of 0.372 absorbancy unit established by the use of sodium PAS will be applicable in determining the MAP content of samples of PAS, inasmuch as a sample weight equivalent to 0.500 Gm. of PAS has been taken in each case, and an amount of NaOH is added which is equivalent to the excess over the amount required to neutralize 0.500 Gm. of PAS. Additional evidence that the equation can be applied in analyzing samples of sodium PAS was obtained by making three additions of MAP to each of four samples of sodium PAS which had been found by this analysis originally to contain 0.02 to 0.04% MAP. The values found upon analyzing these synthetic mixtures were in excellent agreement with those predicted from the sum of the amount originally present and the amount added.

Study of Variations in Conditions.—In order to study the effect of changes in the weight of sample taken on the absorbancy at 440 mμ after development of color, replicate analyses were made on a single lot of medicinal grade PAS. The proposed procedure was followed exactly except that sample weights of 0.050, 0.100, 0.250, 0.500, and 1.000 Gm. were taken. (A sample weight of 0.500 Gm. is used in the proposed procedure.) If the weight of PAS taken does not affect the color intensity, the ratio A_{440} per Gm. PAS taken should be constant. Reference to column 3 of Table II shows that this ratio is not constant; therefore, it is essential for good reproducibility to control the concentration of PAS in the solution being measured. The change in this ratio with changes in PAS concentration may be explained by assuming that the absorbancy values represent a composite of colors: one color is that

[3] In this paper, the standard deviation is calculated according to the following equation:

$$\sigma = \left(\frac{1}{c_2}\sqrt{\frac{\pi}{2}}\right)\frac{\Sigma X}{n}$$

where X is the deviation of a single value from the arithmetical mean, n is the number of values entering into the mean and c_2 is the factor used to correct for the number of values used in calculating the standard deviation. The factor c_2 is given by Shewhart (5) to correct for the small sample bias. He also gives the term $\sqrt{\frac{\pi}{2}}\frac{\Sigma X}{n}$ to normalize the average deviation. This gives a somewhat higher value for the standard deviation for a small sample population than does the conventional equation,

$$\sigma = \sqrt{\frac{\Sigma X^2}{n-1}}$$

formed from MAP and the other that formed by side reactions of PAS.

The addition of constant increments of MAP over the concentration range of interest to PAS samples caused approximately equal increases in absorbancy over a range of PAS concentrations of from 1.8 to 25 mg./100 ml. This indicates that the variations in the ratio in column 3 of Table II are caused by side reactions due to PAS.

TABLE II.—EFFECT OF CHANGE IN WEIGHT OF PAS SAMPLE ON INTENSITY OF COLOR

Wt. PAS Taken, Gm.	A_{430} (Measured)	A_{430} per Gm. PAS Taken
0.050	0.025	0.500
0.100	0.054	0.540
0.250	0.157	0.628
0.500	0.428	0.856
1.000	1.18	1.18

In order to study the effect of aging the dilute NaOH solution of PAS on the value found for the MAP content by the recommended procedure, five portions of 0.500 Gm. each of a sample of medicinal grade of PAS were dissolved in 5 ml. of normal NaOH, as described in the proposed procedure, and then treated with 80 ml. of distilled water. One of these portions was immediately acidified as described in the proposed method, while the other four solutions were allowed to stand for periods of time varying from ten to forty minutes in a water bath held at 28° prior to acidification. Immediately after acidification, each solution was quickly diluted to volume and treated with nitrite and sodium carbonate exactly as described in the recommended procedure. The values found for the MAP content of the PAS sample after aging in the dilute NaOH solution for varying periods of time are given in Table III.

TABLE III.—EFFECT OF AGING THE DILUTE NaOH SOLUTION OF PAS ON THE VALUE FOUND FOR THE MAP CONTENT

Time of Standing in NaOH Soln., Min.	Measured Absorbancy	MAP Content Found, %
Less than 1	0.403	0.04
10	0.389	0.02
20	0.391	0.03
30	0.408	0.04
40	0.424	0.06

The values in Table III indicate that, in the proposed procedure, the sample of PAS may be dissolved in normal NaOH solution and allowed to stand for at least thirty minutes with no significant effect on the value found for the MAP content.

In order to determine the effect of aging the acidified solution of PAS, after the addition of 10 ml. of the (1 + 9) sulfuric acid but prior to the addition of nitrite, on the value found for the MAP content by the proposed procedure, an acidified solution of a sample of medicinal grade PAS was prepared by following the proposed procedure exactly. Then nine aliquots of exactly 5 ml. each of this acidified solution were diazotized and the color developed by following the proposed procedure except that the acidified solution was allowed to stand for periods of time varying from two and one-half to sixty minutes in a water bath at 28° prior to measurement of each 5-ml. aliquot. The values found for the MAP content of the sample after the acidified solution had been allowed to age for various periods of time are given in Table IV.

TABLE IV.—EFFECT OF AGING THE DILUTE SULFURIC ACID SOLUTION OF PAS ON THE VALUES FOUND FOR THE MAP CONTENT

Time of Standing in Dilute Sulfuric Acid, Min.	Measured Absorbancy	MAP Content Found, %
2 to 2.5[a]	0.402	0.03_6
5	0.460	0.10_5
10	0.528	0.18_4
15	0.580	0.24_8
20	0.640	0.30_9
25	0.700	0.39_1
30	0.740	0.43_8
45	0.855	0.57_6
60	0.975	0.71_8

[a] Proposed procedure.

The values in Table IV indicate that aging the sample of PAS in dilute sulfuric acid solution for even a very short time can give rise to a serious error in the MAP content found. These findings are essentially in agreement with those of Oberweger, Seymour, and Simmonite (3), who stated that in acidic solutions the rate of decarboxylation is quite rapid. Therefore, it is essential that the elapsed time between the addition of the 10 ml. of (1 + 9) sulfuric acid to the dilute NaOH solution of the sample and the subsequent addition of the sodium nitrite solution should be kept to a minimum, and also that this time should be approximately the same for each sample which is analyzed. In the course of this work, it has been found that it is possible to add the required amount of (1 + 9) sulfuric acid to the dilute caustic solution of the sample, dilute the acidified mixture to volume, mix well, measure the 5-ml. aliquot into the diazotization flask, and add the sodium nitrite solution in a period of two to two and one-half minutes. It can be seen that, although a systematic error could arise as a result of the decomposition of PAS to MAP during this two and one-half minute period, this error has been compensated for in the proposed method inasmuch as the straight line described by the standard equation for the proposed method has as its intercept on the 0% MAP axis a point which was established by analyzing by the proposed method a sample of sodium PAS which was considered to be free of MAP. The fact that this decomposition is quite constant when the proposed procedure is followed exactly is also evident from the good reproducibility of the values obtained when the proposed method is applied to determining the MAP content of samples of medicinal grade PAS and sodium PAS.

If it is assumed that the increase in absorbancy from zero time to two and one-half minutes is the same as that for two to two and one-half minutes to five minutes, or 0.058 absorbancy unit, then it must be concluded that only a portion of the total correction value 0.372 is caused by the decomposition in acid solution. Other effects not studied may

also be partly responsible for this correction. Four effects which may conceivably be involved are (*a*) coupling of 2,4-dihydroxybenzoic acid to PAS diazo, (*b*) coupling of the diazonium salt of MAP to undiazotized PAS, if such a coupling product is markedly different in absorption from the expected coupling product, (*c*) decarboxylation of the diazonium salt of PAS to give the diazonium salt of MAP, and (*d*) an altered rate of decarboxylation of PAS in the presence of nitrous acid as compared to the rate of decarboxylation in sulfuric acid alone. It must be emphasized, however, that whatever the source of the correction, the reproducibility of the values obtained when the proposed procedure is followed exactly indicates that that effect is more or less constant under the conditions of the proposed method.

TABLE V.—ANALYSES OF COMMERCIAL SAMPLES

Sample	MAP Content, % — Single Values	Average
PAS 1	0.041, 0.069, 0.029, 0.044	0.046
PAS 2	0.021, 0.031, 0.061	0.039
PAS 3	0.138, 0.069	0.104
PAS 4	0.048, 0.035	0.042
Sodium PAS 1	−0.004, 0.004, 0.003, 0.012, −0.008	0.001
Sodium PAS 2	0.004, −0.001, 0.007, 0.009	0.005
Sodium PAS 3	0.016, 0.012, 0.019, 0.020, 0.006	0.015
Sodium PAS 4	0.017, 0.022, 0.009, 0.006, 0.022	0.015

An obvious improvement in the proposed method would be a modification whereby acidification of the solution of the sample would be postponed until after the addition of the sodium nitrite solution, so that the aging of the sample of PAS in dilute sulfuric acid solution would be further minimized. This would require establishing a new correction under the new conditions.

Precision and Accuracy.—The precision of a single value for the MAP content of a sample of PAS, based upon 86 single determinations by the proposed method performed on 30 samples of PAS on different days, and calculated in terms of % MAP, may be expressed by a standard deviation of ±0.019% MAP. The precision of a single value for the MAP content of a sample of sodium PAS, based upon 52 single determinations by the proposed method performed on 16 samples of sodium PAS on different days, and calculated in terms of % MAP, may be expressed by a standard deviation of ±0.008% MAP. The reason for the improved precision when the proposed method is applied to samples of sodium PAS, as compared with that for samples of PAS, is unknown. There is no reason to believe that the proposed method is subject to any errors other than its own random errors if the sample of sodium PAS which was used to establish the correction applied in the standard equation is considered to be free of MAP.

Table V gives a few typical examples of the kind of replicate values which have been obtained.

Analyses of Commercial Samples.—The MAP contents of 30 samples of medicinal grade PAS from various commercial sources, both domestic and foreign, were found by the proposed method to range from 0.01% MAP to 0.20% MAP. The MAP contents of 16 similar samples of medicinal grade sodium PAS ranged from 0.00% MAP to 0.11% MAP.

BENZOYL PEROXIDE REACTION[1]

Previous to the publication of Pesez's method (1)[2] the authors of this paper had devised and made considerable progress on a solution of the same problem by a different approach; namely, by oxidizing MAP in the presence of PAS to form a brown color which could be measured. It was found that a controlled oxidation at a temperature of 35° of a solution of the product in acetone by means of a solution of benzoyl peroxide in benzene would produce such a color. A correction had to be applied for some decarboxylation of PAS to MAP which occurs in the course of the analysis and which affects the final value. The details of the benzoyl peroxide method were worked out so that it was possible to obtain MAP values in the region below 1% MAP with a standard deviation of ±0.025% MAP. It was found, however, that a small amount of color is formed from some source other than from the MAP which is either present as such or is produced by the decarboxylation of PAS. It became necessary, therefore, ultimately to standardize this method by means of PAS samples analyzed by the diazotization and coupling method. In addition, it could not be used for sodium PAS. Since the method is not recommended as an analytical procedure, in view of the superiority of the proposed method, it is not to be discussed any further in this paper. The benzoyl peroxide reaction did, however, bring out some points which may throw some light on the problem of the stability of PAS with respect to discoloration. A separate paper bearing on these facts will, therefore, be submitted for publication in the near future

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An Evaluation of Banthine in Hyperhidrosis*

By ARTHUR G. ZUPKO†,‡

Forty-four subjects with varying degrees of hyperhidrosis and four normal subjects were given daily doses of Banthine bromide (*beta*-diethylaminoethyl xanthene-9-carboxylate methobromide) over a period of four to six months. A few selected subjects were also given placebos at irregular intervals. A complete hematological study, electrocardiogram (Lead II), and basal metabolic rate were done on each subject before administration of the drug and at intervals during the study. Results indicate that the hematopoietic system is unaffected, and that the basal metabolic rate and electrocardiogram tracing deviate little from the normal. Some side reactions appear concomitant with administration of the drug, and in a few cases a tolerance develops. Banthine bromide effectively controls the hyperhidrosis as long as administration of the drug is continued. The limitations of the drug are discussed.

BANTHINE BROMIDE (*beta*-diethylaminoethyl xanthene-9-carboxylate methobromide) is a quaternary amine which has been used with much success in the treatment and control of peptic ulcer. Pharmacological studies indicate that Banthine possesses both an anticholinergic and autonomic ganglionic blocking action (1, 2).

During the course of treating a group of 144 patients with peptic ulcer and other gastrointestinal disorders, Grimson and his co-workers noted the effectiveness of Banthine in suppressing excessive sweating in several patients. Subsequently they treated four patients with pronounced hyperhidrosis and obtained effective control with Banthine (3). Since sympathectomy has proved to be an unsatisfactory treatment for hyperhidrosis, Grimson suggested that perhaps Banthine would effectively control this condition.

In order to evaluate further the efficacy of Banthine in the control of hyperhidrosis it seemed desirable to observe the effects of the drug on the hematopoietic system, on basal metabolism, and on the cardiac mechanism when administered to a large group of subjects over a prolonged period of time.

EXPERIMENTAL

Methods

Forty-four volunteer subjects with varying degrees of hyperhidrosis and four normal subjects were selected as the test group. The age, sex, weight, and height, as well as the duration and severity of the hyperhidrosis of the subjects are shown in Table I. All but one of the subjects were below 40 years of age, with an average age of 25.1 years. Thirty-nine males and nine females were included in the study. The duration of the symptoms in the subjects varied from a few years to as long as could be remembered. The severity of the hyperhidrosis ranged from mild sweating of the hands, feet, groin, axillae, and perineum to maceration of the skin of the hands and feet.

Prior to administration of the drug each subject was given an information sheet explaining the procedure to be followed during the ensuing weeks. The routine consisted of a preliminary hematological examination, basal metabolism, and electrocardiogram. Upon completion of the tests each subject was given an envelope containing enough Banthine bromide for a week of medication. Ample direction regarding administration and admonition of the side effects were given. The subjects reported each week for an additional supply of the drug and related their experiences with the medication for the previous week. The hematological examination, basal metabolism, and electrocardiogram were repeated every five to seven weeks.

The hematological examination consisted of a hemoglobin determination, erythrocyte, leukocyte, and differential counts. The basal metabolic tests were done with a McKesson Metabolor Model No. 185. Subjects refrained from eating, drinking, and smoking from midnight to the time of the morning test. The electrocardiograms (Lead II only) were taken with a portable direct-writing McKesson Cardiofax.

In mild cases a 50-mg. tablet of Banthine bromide was taken orally in the morning, one in the afternoon with an additional dose in the evening if the subject had any social engagement. In the more severe cases the dosage was increased proportionately but never exceeded 200 mg. per day.

The four control subjects were given one 50-mg. tablet of the drug to take in the morning and one in the evening. Both groups of subjects continued the daily medication for four to six months.

Results

Charts were maintained for each subject, showing results of preliminary tests, dosage level, and findings after medication. A typical chart (subject 1) is presented in Table II.

Effect on Basal Metabolism.—Prior to the administration of Banthine bromide, 24 of the 44 subjects with hyperhidrosis had normal basal metabolic rates (±10%), whereas 13 had metabolic rates

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TABLE I.—PERTINENT DATA ON TEST SUBJECTS

No.	Age	Sex	Weight, Lb.	Height, In.	Duration, Yr.	Severity of Symptoms
1	24	M	160	73	5	Pronounced: hands, feet, axillae involved no skin maceration; perineum moist.
2	31	F	120	65	10	Mild: hands, axillae moist and clammy—feet unaffected.
3	20	M	200	68	Life	Mild: hands and feet very moist, axillae very dry.
4	42	M	210	69	8	Severe: slight skin maceration of the right hand; feet and axillae soggy and wet.
5	32	M	132	64.5	7	Pronounced: hands, feet, axillae glistened with moisture; groin sweaty.
6	25	M	150	68	4	Mild: hands and feet only area affected.
7	28	M	174	70.5	12	Pronounced: hands, feet, axillae involved; extremities very cold and wet.
8	28	M	142	68	Life	Mild: feet only area of body affected (wet, cold, and clammy).
9	30	M	175	69	Unknown	Mild: hands and feet cold, damp; no axillary involvement.
10	38	M	170	70.5	20	Mild: palms of hands only area affected.
11	24	M	145	69.5	Life	Severe: foot tissue soggy and soft to touch; pronounced sweating on hands and axillae.
12	23	M	150	71	Unknown	Mild: feet only area affected.
13	29	M	174	66	Life	Pronounced: hands, feet, axillae affected (drenched); perineum and groin moist.
14	21	M	158	65	Life	Mild: hands and feet glistened with moisture.
15	22	M	185	71.5	Life	Mild: hands and feet glistened with moisture.
16	20	M	190	74	10	Mild: hands only area affected.
17	30	M	158	68	12	Pronounced: hands, feet, axillae drenched with perspiration.
18	18	F	123	62	Life	Mild: hands only area involved.
19	29	M	225	75.5	10	Mild: hands, feet, axillae affected.
20	23	M	155	69	4	Pronounced: hands and feet very wet and clammy; axillae quite moist; perineum very moist.
21	32	M	215	75	Life	Mild: axillae very moist; feet and hands moderate.
22	21	F	120	66	Life	Mild: hands cold and clammy, wet; feet and axillae not affected.
23	20	M	285	75.5	Life	Mild: palms of hands and axillae only area affected.
24	20	M	135	71	Life	Mild: hands, feet, axillae affected; slight groin involvement.
25	35	F	158	69	8	Pronounced: axillae very moist continuously; hands and feet soggy.
26	33	M	137	69	Life	Mild: hands and feet very moist; axillae unaffected.
27	23	M	170	67	Life	Mild: hands, feet, axillae involved.
28	30	M	188	72	13	Mild: hands, feet very moist; axillae unaffected.
29	23	M	173	70.5	Life	Pronounced: hands and feet soggy and soft to touch; axillae very wet.
30	19	M	147	69	Life	Mild: feet and hands cold, clammy and wet; axillae normal.
31	18	M	150	72	Life	Pronounced: continuous perspiration on hands, feet, axillae and perineum.
32	18	F	115	62	Life	Mild: hands dry, feet excessively wet; axillae dry.
33	18	M	151	71	Life	Pronounced: hands, feet, axillae continuously wet; perineum involved.
34	17	M	158	71.5	5	Mild: hands and feet glistened with moisture.
35	18	M	150	70	6	Mild: palms of hands mainly affected.
36	23	M	180	74.5	5	Mild: feet and hands cold and wet; axillae dry.
37	23	M	190	72	Life	Mild: palms of hands involved only.
38	22	M	160	70.5	5	Mild: hands and feet very wet; axillae very dry.
39	27	F	108	62	11	Pronounced: excessive sweating of hands, feet, and axillae.
40	23	M	142	72	Life	Pronounced: palm of left hand very soggy; feet and axillae extremely wet.
41	20	M	145	68	Life	Mild: palms of hands mainly affected; some groin involvement.
42	39	M	213	71	14	Mild: feet and hands wet and cold.
43	22	F	110	62	3	Pronounced: continuous sweating of hands, feet, and axillae.
44	28	F	128	64	15	Mild: palms of hands only involvement.
Controls						
C45	24	M	142	70	None	None
C46	20	F	105	62	None	None
C47	29	M	164	72	None	None
C48	26	M	155	69	None	None

above 10% (14 to 32%), and in seven the basal metabolic rate was less than −10% (−15 to −38%). After a varying number of weeks of daily ingestion of the drug, only one of the 24 subjects with a normal BMR showed any change. This subject gave consistent readings of +18 to +25%, whereas before ingesting the drug his BMR was −3%. Four weeks after administration of the drug ceased, the BMR returned to normal limits. None of the 13 subjects with basal metabolic rates above +10% was

affected beyond a ±5% change. Only one of the seven with a BMR below −10% showed any appreciable change. This subject had a preliminary BMR of −17% and after fifteen weeks of medication showed a +9% on two successive tests taken one week apart. Five weeks after stopping medication the BMR was still on the plus side.

All four control subjects had a normal BMR previous to taking the medication. No appreciable change was noticed after sixteen to eighteen weeks of daily doses of Banthine bromide.

Effect on the Hematopoietic System.—Preliminary examinations of the subjects revealed that three had a hypochromic anemia with a color index of 0.71, 0.69, 0.76, respectively, and two had a hyperchromic anemia with a 1.15 and 1.21 color index. The remaining 39 subjects were within normal limits. In the latter group only two significant changes were observed after twelve to fifteen weeks of medication. Both of the subjects showed a pronounced decrease in neutrophilic granulocytes and an increase in lymphocytes, but no appreciable change in the total leukocytic count.

In the group of five anemic subjects the two with hyperchromic anemia showed a lowering of the color index during the course of the experiment and the three hypochromic subjects increased their color index. All five were under treatment for anemia. None of the five showed any leukocytic changes. It is interesting to note that the Banthine bromide did not interfere with the expected improvement in the subjects under treatment for anemia.

The four control subjects were within normal hematological limits both before and after ingestion of the drug.

Effect on Cardiac Mechanism.—The ingestion of Banthine bromide did not significantly alter the rate or rhythm of the heart at the dosage levels necessary to control hyperhidrosis in the subjects under study. The Lead II measurements of the various waves and P-R, QRS, Q-T, S-T intervals were within the normal values set forth by Burch and Winsor (4) in all except four subjects. Before medication three of these showed a sinus arrhythmia with no evidence of heart block and one a sinus bradycardia. Daily ingestion of the drug over many weeks resulted in little change from the preliminary observation in these four subjects.

Effect on Hyperhidrosis.—As noted in Table I the 44 hidrose subjects were arbitrarily classified into individuals with mild, pronounced, and severe hyperhidrosis depending upon the severity of the symptoms. Of 29 subjects classified as mild cases, 27 showed very definite improvement in the form of adequate control of perspiration with the daily ingestion of two 50-mg. tablets of Banthine bromide, an additional tablet being taken in the evening whenever necessary. One of the subjects in this group obtained no relief even when the dosage was increased to 200 mg. daily. The remaining subject had to discontinue the medication because of excessive dryness of the mouth.

All 13 of the subjects with pronounced hyperhidrosis obtained adequate control with daily doses of three 50-mg. tablets of the drug and whenever needed, an additional dose in the evening. The two subjects with severe hyperhidrosis took daily doses of 200 mg. of the drug. This amount had no effect upon either subject in reducing the excessive sweating.

As a rule dryness of the affected parts usually began from one-half to one hour after ingestion of the drug and persisted for about four hours. In four cases it appeared that a tolerance to the drug developed. In these subjects the dryness lasted for about four hours during the first six weeks of medication but in later weeks little relief was obtained. Even when the dosage was increased the same pattern ensued.

Side Effects.—The side effects of Banthine bromide have previously been reported by several investigators (1, 5, 6). These consist mainly of a dryness of the mouth, bitterness of taste, tendency toward constipation, dysuria, pupillary dilatation, blurred vision, and production or accentuation of a tendency toward heartburn. Table III shows the side effects reported by the test subjects at the dosage level used for control of the hyperhidrosis by each subject. The bitter taste and dryness of the mouth are purposely omitted, since all the subjects experienced these effects to some degree. In all but one case these two effects gradually wore off, or the subject became adjusted to them. Excessive dryness of the mouth necessitated discontinuance of the drug in the one instance cited previously.

Side effects after Banthine bromide were reported by 23 subjects with hyperhidrosis and 3 control subjects of a total number of 44 hidrose and 4 normal individuals. In all instances, with the exception of 2 in the hidrose group, the side effects were mild or disappeared within a short time. Persistent headache in one patient and repeated constipation in another necessitated discontinuance of the drug. Hyperhidrosis in both of these patients was controlled when Banthine was administered.

DISCUSSION

Those subjects with persistent complaints of side effects were given placebos containing 0.2 mg. of atropine and a small amount of quinine to simulate the side effects and taste, respectively, of Banthine bromide. Relief from the most common complaints of constipation, headache, and heartburn was reported after substitution of placebos for Banthine. In the few subjects who obtained no effective control of excessive sweating with Banthine, administration of the placebos was not influential in altering the negative response of this group. Only one of all the subjects afforded relief from hyperhidrosis with Banthine claimed similar results with placebos. The results with the use of placebos indicate the accuracy of the subjective reports by the test group.

Although Banthine effectively controlled the hyperhidrosis in 40 of the 44 test subjects, it is necessary to point out that whenever the drug was temporarily discontinued the excessive sweating reappeared with regularity. It becomes apparent that administration of the drug must be continued for an indefinite period if the hyperhidrosis is to be kept under control.

The extreme bitterness of the compound will influence many individuals to submit to the discomfort and embarrassment of hyperhidrosis rather than continue prolonged medication with Banthine. A more palatable dosage form is now being devised

TABLE II.—TYPICAL CHART OF A HYPERHIDROSIS SUBJECT

Preliminary Tests			Dosage,	5th Week			12th Week			19th Week		
BMR, %	ECG, Sec.	Hematology	50-Mg. Tabs.	BMR, %	ECG, Sec.	Hematology	BMR, %	ECG, Sec.	Hematology	BMR, %	ECG, Sec.	Hematology
+15	P − R = 0.20s	Hb% 93.7	t. i. d.	+18	P − R = 0.20s	Hb% 95.1	+20	P − R = 0.20s	Hb% 94.6	+16	P − R = 0.20s	Hb% 94.9
	QRS = 0.08s	RBC 5,265,000			QRS = 0.08s	RBC 5,200,000		QRS = 0.07s	RBC 5,180,000		QRS = 0.09s	RBC 5,275,000
	QT = 0.359s	WBC 7,000			QT = 0.363s	WBC 8,500		QT = 0.371s	WBC 8,250		QT = 0.368s	WBC 7,950
	ST = Isoelec.	Differential %: Neutrophils—66			ST = Isoelec.	Diff: N—62		ST = Isoelec.	Diff: N—61		ST = Isoelec.	Diff: N—68
	Rate: 78/min.	Eosinophils—3			Rate: 80/min.	E—4		Rate: 78/min.	E—3		Rate: 80/min.	E—2
	Normal sinus rhythm	Basophils—0			Normal sinus rhythm	B—1		Normal sinus rhythm	B—0		Normal sinus rhythm	B—1
		Lymphocytes—27				L—28			L—32			L—27
		Monocytes—3				M—5			M—4			M—2
		Unknown—1				U—0			U—0			U—0

TABLE III.—SIDE EFFECTS AFTER BANTHINE BROMIDE

Side Effect	Number	Per Cent
Constipation	9	18.8
Dysuria	2	4.2
Heartburn	8	16.7
Headache	12	27.1
Mydriasis	3	6.3

by the manufacturer in an effort to overcome this disadvantage.

Daily administration of the drug over a period of several months had no effect upon menses in the nine female subjects.

SUMMARY

1. Daily doses of Banthine bromide were given to 44 volunteer subjects and four normal subjects over a period of four to six months. The medication effectively controlled the hyperhidrosis in approximately 90 per cent of the test subjects.

2. The hematopoietic system, basal metabolism, and cardiac mechanism were unaffected by Banthine at the dosage level necessary to control hyperhidrosis in the individual subject.

3. Some side reactions, notably constipation, headache, and heartburn, appeared concomitant with administration of the drug in both the hidrose and normal subjects. Employment of similar-appearing placebo tablets substantiated these observations.

4. Four of the 40 subjects in whom Banthine effectively controlled the hyperhidrosis developed a tolerance to the drug after six weeks of medication.

5. Surreptitious use of the drug should be discouraged because of the side effects and possible toxic manifestations in individuals with pyloric or bladder obstruction, prostatic enlargement, glaucoma, impaired vision, and those with a tendency toward constipation or heartburn. The drug should not be dispensed except on the advice of a physician.

6. Banthine bromide effectively controls hyperhidrosis in a majority of cases but administration of the drug must be continued indefinitely.

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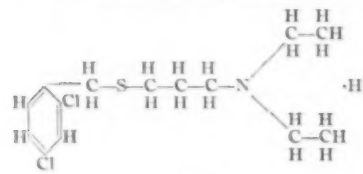
A Pharmacological Study of N,N-Diethyl-[5-(2,4-Dichlorophenyl)-4-Thiapentyl] Amine Hydrochloride*

By P. V. HAMMOND† and L. D. EDWARDS

Preliminary tests indicate that N,N-diethyl-[5-(2,4-dichlorophenyl)-4-thiapentyl] amine hydrochloride acts chiefly as a spasmolytic and as a local anesthetic. Tests for antihistaminic and analgesic activity were essentially negative.

TWO SERIES of sulfur compounds related to Benadryl and Pyribenzamine in which sulfur replaces an oxygen or nitrogen of the prototype molecules have been reported by Boenigk, Christian, and Jenkins (1). These compounds were made available to this laboratory for pharmacological testing. Preliminary tests indicate that one of these compounds, N,N-diethyl-[5-(2,4-dichlorophenyl)-4-thiapentyl] amine hydrochloride, possesses considerable activity as a spasmolytic and as a local anesthetic. This compound (hereafter termed compound No. 6) is a light, white, odorless, water-soluble powder melting at 116–117°. Its chemical structure is as follows:



EXPERIMENTAL

Spasmolytic Action

(*a*) Modification of contractions predominately due to longitudinal muscles of the guinea-pig ileum: Using terminal guinea-pig ileum strips in 75 cc. of aerated tyrode solution maintained at 38°, contractions produced by spasmogenic agents (histamine, mecholyl, and barium chloride) before and after compound No. 6 were obtained. In all cases compound No. 6 was permitted to act two minutes before the second addition of the spasmogenic agent. Using 7.5 μg. of histamine phosphate as the spasmogenic agent, the minimal effective bath concentration of compound No. 6 to show significant spasmolytic action was 1:450,000. Against 5 mg. of barium chloride the minimum effective concentration was 1:100,000, and in the case of 7.5 μg. of mecholyl it was 1:450,000.

(*b*) Modification of contractions predominately due to circular muscles of the rat jejunum: The *in vitro* technique to record contractions of the circular muscles of intestinal strips (2) used in this phase of this study involved the use of a special cannula approximating a U-shaped connecting tube. One arm of the tube was cut and fashioned into a movable cannula to support the intestinal strip, while the other arm was connected to a tambour. The stem arm was inserted into a rubber stopper which formed the bottom of the bath, and provided a means of introducing liquids into the lumen of the strip. With the lumen pressure, temperature, tension, *p*H, and ions present controlled, a normal pattern of a peristaltic type of contraction could be recorded. In the rat the normal contractions were of a constant magnitude and occurred with strikingly constant frequency. In the results reported in Table I, Locke-Ringer (minus the sodium bicarbonate and dextrose) solutions of the drugs were placed in the lumen of the segment after normal contractions had been recorded. The effective concentration of a drug in the lumen is higher, as a rule, than when the drug is placed directly in the bath. It is to be noted that compound No. 6 in a lumen concentration of 1:600,000 materially decreased the height of the contractions and lengthened the time between contractions without completely abolishing the contractions. This reduction of the height of the contractions and the increase of the time between contractions is considered as further evidence of spasmolytic activity.

Local Anesthetic Action

Compound No. 6 was screened for local anesthetic activity by means of the guinea-pig wheal method (3) and the rabbit cornea method (4). In the case of the guinea-pig wheal method 0.25 cc. of a 1% solution of the drugs was used as the test dose, and a tetanizing current as the stimulus. The average results are given in Table II.

On the rabbit's cornea using a 1:1,000 solution of compound No. 6 the average onset (10 animals) was 9.10 ± 0.91 (S. E.) minutes and the average duration was 17.2 ± 2.68 (S. E.) minutes. Papaverine and pavatrine in the same concentration did not exhibit any local anesthetic activity with this method. In the compound No. 6 tests, signs of irritation were noted.

Toxicity Studies

The twenty-four-hour LD_{50} as determined by intravenous injection into a tail vein of female Wistar rats (weight range 120–190 Gm.) and calculated by the DeBeer nomograph method (7) was 28.33 mg./Kg. (limits of error 90.6 to 110.3%). A group of 10 animals was used for each dose—20, 25, 30, 35, and 40 mg./Kg. The toxic symptoms included tonic convulsions and respiratory depression. Death, if it occurred, was, as a rule, early in the twenty-four-hour period of the test.

The daily oral administration of 50% of the above LD_{50} over a period of thirty days and the intraperi-

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TABLE I.—MODIFICATION OF CONTRACTIONS PREDOMINATELY DUE TO CIRCULAR MUSCLES OF THE RAT JEJUNUM BY COMPOUND NO. 6

Drug	Lumen Concn. of Drug	No. of Tests	Average Height of Contractions, Mm.		Average Interval Between Contractions, Mm.		Time to Abolish Contractions, Min.
			Normal	After Drug	Normal	After Drug	
Compd. No. 6	1:600,000	2	35	18	15	38	..
Compd. No. 6	1:100,000	10	21	14	22	48	16
Compd. No. 6	1:75,000	6	30	17	15	40	5
Compd. No. 6	1:60,000	6	49	10	25	38	16
Compd. No. 6	1:50,000	10	31	20	19	67	16
Compd. No. 6	1:40,000	10	49	20	20	40	9
Compd. No. 6	1:25,000	10	39	26	16	21	8
Compd. No. 6	1:10,000	6	32	..	43	..	0
Pavatrine	1:25,000	6	48	42	30	27	3
Pavatrine	1:10,000	6	48	..	36	..	0
Papaverine	1:75,000	6	75	63	21	22	32
Papaverine	1:50,000	6	33	17	23	24	14
Papaverine	1:25,000	6	38	24	21	27	10
Papaverine	1:10,000	6	61	..	19	..	0

TABLE II.—LOCAL ANESTHETIC ACTION OF COMPOUND NO. 6, PAPAVERINE, AND PAVATRINE AS DETERMINED BY THE GUINEA-PIG DERMAL WHEAL METHOD

Drug	No. of Animals	Av. Threshold Stimulus, Cm. ± S. E.	Av. Onset of Anesthesia, Min. ± S. E.	Av. Duration of Anesthesia, Min. ± S. E.
Compound No. 6	9	5.44 ± 0.50	1.55 ± 0.17	23.88 ± 1.81
Papaverine	10	2.35 ± 0.02	2.00 ± 0.00	15.60 ± 1.00
Pavatrine	10	2.70 ± 0.006	1.91 ± 0.09	25.00 ± 3.55

$$\text{S. E.} = \sqrt{\frac{(y - \bar{y})^2}{n(n-1)}}$$

toneal injection of 25% of the LD_{50} over a period of sixty-two days to Wistar rats of either sex (weight range 150–200 Gm.) did not show any significant gross or microscopic pathology.

Miscellaneous Activity Tests

Compound No. 6 given intraperitoneally in doses up to 40 mg./Kg. to guinea pigs afforded little or no protection against a histamine aerosol. Using the Davies, Rarentos, and Walpole method (5) intraperitoneal doses up to 7.8 mg./Kg. to rats did not show significant analgesia. A concentration of 1% was required to inhibit *Staphylococcus aureus* and *Escherichia coli* cultures (wet filter paper technique). When injected subcutaneously into the rabbit's ear, 0.5 to 2% solutions of compound No. 6 produced an irritant effect comparable to that seen with equal concentrations of sodium nitrate (6).

CONCLUSIONS

1. Preliminary tests indicate that N,N-diethyl-[5-(2,4-dichlorophenyl)-4-thiapentyl] amine hydrochloride (compound No. 6) acts chiefly as a spasmolytic and as a local anesthetic.

2. Tests for antihistaminic and analgesic activity were essentially negative.

3. The LD_{50} determined intravenously in the white rat is 28.33 mg./Kg. (limits of error 90.6 to 110.3 per cent). Repeated doses, oral and intraperitoneal, did not show in the white rat any significant gross or microscopic pathology.

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A Preliminary Phytochemical Study of *Lithospermum ruderale* Douglas*

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It has been confirmed that anestrous is produced in mice by the ingestion of *Lithospermum ruderale* Douglas. Extraction studies and a preliminary phytochemical study are included in this report.

LITHOSPERMUM RUDERALE Douglas Fam. *Boraginaceae* (1) is an herb that is indigenous to some of the Rocky Mountain states, i. e., Montana, Nevada, Utah, Idaho, and Wyoming. It is commonly known by the name "stoneseed" but it has other common names and synonyms which were given to it by various Indian tribes who used it as a medicinal herb.

Among most of the Shoshones (2), the root of the plant was a favorite remedy for diarrhea. In one settlement, it was believed that the plant had contraceptive properties. Later reports (3, 4) have stated that when this plant was fed to adult female mice, with previously regular estrous cycles, the mice developed prolonged periods of diestrous. More recently (5) it has been demonstrated that this plant decreases the gonadotropic potency of the pituitary glands of adult female mice, without producing irreversible or histological damage to the pituitary gland.

Since a survey of the literature revealed no report of chemical study of this plant, this investigation was carried out in an attempt to contribute to the chemistry of this drug.

EXPERIMENTAL

Preparation of the Plant Material.—The plant material used in this study consisted of the air-dried aerial portion of the drug, free of adhering foreign material. A sample of this herb was identified as *Lithospermum ruderale* Douglas of the *Boraginaceae* family by Dr. S. F. Blake of the United States Department of Agriculture, Bureau of Plant Industry, Beltsville, Md.

The plant material was ground in a Jacobson laboratory mill to a No. 20 powder. The ground material, weighing approximately 12 Kg., was stored in tightly closed metal cans. The ground material was light green in color, and had a characteristic odor.

Pharmacological and Extraction Studies.—In order to determine whether or not the plant material used in this study had antiestrous activity, the whole ground plant was fed to mice at a level constituting 30% of their diet. The mice went into diestrous for the period of time they were on this diet. At a lower drug concentration, diestrous was produced, but the results were more erratic.

Four kilograms of ground plant material was percolated with Skelly-B in a continuous Soxhlet extractor. A portion of this defatted plant material was fed to mice at a 30% diet level. The mice developed immediate and continuous diestrous.

The defatted marc, which weighed 3,600 Gm., was cold-percolated with 95% ethyl alcohol. The percolate was concentrated as it was collected, by distillation at reduced pressure at a temperature not above 55°. As the extract became more concentrated, some crystalline material separated out, and was shown to be inorganic. Qualitative tests indicated that the compound was potassium chloride. The residue which remained when almost all of the solvent had been removed was stirred with 1,500 cc. of distilled water, and the resulting mixture separated by centrifuging. The resulting aqueous fraction, which was brown in color, was concentrated to 600 cc. Some antiestrous activity was observed when this extract was fed at a diet level which represented 60% of the crude drug.

As the water fraction became more concentrated, some yellow material crystallized out. About 20 Gm. of impure greenish-yellow material was obtained from the water fraction. This material had no antiestrous activity.

The water-insoluble fraction was dissolved in chloroform. This fraction was tested next, but produced no antiestrous response.

Since none of these fractions of the material that had been extracted from the plant with 95% alcohol showed marked activity, the marc was tested and failed to exhibit antiestrous activity at a 30% diet level.

A portion of ground, defatted plant material was macerated with distilled water, and subsequently percolated with 50% alcohol. The first, or aqueous, portion of the percolate was brown colored; however, the subsequent percolate, which comprised the bulk of the extract, was green. This percolate was concentrated under reduced pressure at a low temperature. This extract proved to be inactive.

An attempt was made to obtain an aqueous extract of the plant material. However, the drug absorbed so much water and the mixture was so mucilaginous that it was necessary to add sand and Filter-Cel to the wet mass. Chloroform was added to the aqueous percolate as it was collected. This percolate was concentrated by distillation of the solvent at a low temperature and pressure. This extract was inactive at a 30% diet level. An alco-

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§ We are indebted to Dr. E. M. Cranston for doing the pharmacological testing.

holic extract (95%) and a hot water extract were made of this marc which had been extracted with cold water, but neither extract was active.

Five hundred grams of defatted powdered plant material was wet with distilled water and then placed in a tray to dry. The plant material turned dark brown when it became wet. When the plant material had dried, it was macerated with 95% alcohol and percolated with this same solvent at a slow rate. This extract, which was concentrated under reduced pressure, had no antiestrous activity.

One hundred and fifty grams of powdered defatted plant material was refluxed for two hours with 800 cc. of 95% ethanol containing 2 Gm. of tartaric acid. The alcoholic solution was strained from the marc and the alcohol was nearly all removed by distillation. The concentrated alcoholic solution was recombined with the marc, and this recombined material was allowed to dry in the air. This material produced some antiestrous response, but, since the tests were made on only five mice, the results were not conclusive.

Pharmacological testing was concluded at this point, since the mice which were being used for these tests were getting too old to give reliable results, and fresh mice were not readily available.

A review of these extraction studies leads one to believe the possibility that the antiestrous activity of this drug is not due to a single component, but to two or more factors within the plant, one of which is soluble in alcohol and the other of which is not.

Tests for Alkaloids.—A portion (50 Gm.) of the powdered plant material was shaken for one hour with a mixture of diethyl ether, chloroform, alcohol and ammonia water. The mixture was then allowed to macerate for twenty-four hours, after which the solvent was removed by filtering. The filtrate was then shaken with three successive portions of 2% sulfuric acid in a separating funnel. The acid extracts were combined, mixed thoroughly, and tested with various alkaloidal reagents. Mayer's reagent T. S., tannic acid T. S., and picric acid T. S. all failed to give a positive test or reaction.

Test for Glycosides.—The Stas-Otto procedure (6) was used as the basis for the qualitative analysis for glycosides. A mixture of powdered plant material and 95% alcohol containing tartaric acid was refluxed for eight hours and then filtered. The marc was washed with four successive portions of hot 95% alcohol, and finally the marc was pressed as free as possible of alcohol. The combined filtrate was concentrated to a small volume by distillation, and then evaporated to dryness on a steam bath. Three portions of distilled water were used to wash the dried extractive, and the washings were then combined. This aqueous solution was extracted with diethyl ether in divided portions. The combined ether extract was evaporated to dryness leaving a yellow residue. Sufficient 95% alcohol was added to this residue to effect solution. The Molisch test was applied to a portion of this solution, which proved to be negative.

Isolation of "Wax A" and "Wax B."—Four thousand grams of the powdered plant material was exhaustively extracted with Skelly-B. During this extraction process, a waxy material deposited in the flasks containing the solvent. The petroleum benzin extract was cooled and filtered, leaving a residue of 80 Gm. of waxy material. "Wax A" was obtained by repeatedly boiling the waxy residue with 95% alcohol, filtering, and cooling. Wax A was purified by repeated recrystallization from boiling 95% alcohol.

A dark brown waxy residue remained after Wax A had been extracted with boiling 95% alcohol. This residue was dissolved in boiling isopropyl ether, filtered, and, on cooling, a light brown waxy substance was obtained. This material was further purified by crystallization from hot ethyl acetate and decolorizing with charcoal. A white substance was obtained which was labeled "Wax B."

Wax A.—This substance was insoluble in cold concentrated sulfuric acid and in 5% aqueous sodium hydroxide solution. A chloroform solution of this wax did not decolorize a chloroform solution of bromine. It melted at 71–72°. The average saponification number of this wax was 104.4.

Saponification of this wax with alcoholic potassium hydroxide yielded an acid and an alcohol. Both acid and alcohol were recrystallized from isopropyl ether. The acid melted at 75° and the alcohol at 71–72°. The average neutral equivalent of the acid was 400.15. The phenylurethane of the alcohol was prepared with phenyl isocyanate and petroleum benzin. The phenylurethane was recrystallized from petroleum benzin and melted at 83°.

Wax B.—This wax was insoluble in cold concentrated sulfuric acid, and in 5% aqueous sodium hydroxide solution. A chloroform solution of this wax did not decolorize a chloroform solution of bromine. It melted at 78°. The average saponification number of this wax was 85.5.

Saponification of this wax with alcoholic potassium hydroxide yielded an acid and an alcohol. Both acid and alcohol were recrystallized from isopropyl ether. The acid melted at 76–77° and the alcohol at 74°. The average neutral equivalent of the acid was 382. The phenylurethane was recrystallized from petroleum benzin and melted at 87°.

Orange Pigment.—The alcoholic extract from approximately 4 Kg. of powdered defatted plant material was concentrated to a small volume by distilling off the alcohol under reduced pressure. This concentrated extract was stirred with about 1,500 cc. of warm distilled water. The aqueous fraction was concentrated to about 600 cc. and cooled in a refrigerator. A considerable amount of yellow material precipitated from the solution, and was filtered off and dried. Fractional crystallization of this material was attempted, using hot diluted alcohol. A small quantity of orange-colored material crystallized out first. Several recrystallizations from diluted alcohol gave orange, rosette-shaped crystals which melted at 140°.

The following qualitative tests were applied to the above crystalline material:

Ferric chloride test solution gave a deep green color. The solution did not give a play of colors when made alkaline with sodium carbonate solution added dropwise.

It was soluble in warm glacial acetic acid, producing a yellow-orange solution upon the addition of concentrated sulfuric acid.

It was soluble in concentrated hydrochloric acid, giving a deep orange solution. Upon warming this solution on a steam bath, the color deepened and an orange precipitate formed almost at once.

A negative Molisch test was obtained.

A positive reduction reaction (7) with magnesium and hydrochloric acid, as evidenced by the appearance of a wine-red color, was obtained. This indicated that a flavonol or flavonone type structure was present.

A positive Wilson's boric acid test (8) was obtained. This indicated a hydroxyl group in the 5-position of the flavonol type compound.

A positive test for the methylenedioxy group (9) was obtained.

No methoxyl groups were present in this compound when determined by the Viebock and Schwappach method (10).

Elementary analysis of this orange pigment indicated the absence of sulfur, nitrogen, and halogen.

Anal.—Found: C, 50.6, 50.7; H, 5.26.

Yellow Pigment.—The alcoholic solution, from which the orange pigment had crystallized, was allowed to cool further, whereupon a larger quantity of pale greenish-yellow material crystallized out. This material was washed with cold distilled water, dried, and washed with cold chloroform. Several recrystallizations from diluted alcohol gave pale greenish-yellow rosette-shaped crystals which melted at 178–179°. This substance was soluble in alcohol, acetone, and chloroform.

The following qualitative tests were applied to the above crystalline material:

Ferric chloride test solution gave a deep green color. The addition of a dilute solution of sodium carbonate caused no play of colors.

It was soluble in sodium hydroxide test solution, giving a lemon-yellow color. Acidulation discharged the yellow color and a yellow precipitate formed.

It was soluble in glacial acetic acid, and upon the addition of concentrated sulfuric acid, a deep yellow color was obtained.

It was soluble in both concentrated sulfuric and hydrochloric acids, giving an intense yellow color.

A negative Molisch test was obtained. A positive reduction reaction (7) with magnesium and hydrochloric acid was obtained, as indicated by the appearance of a wine-red color. A positive Wilson's boric acid test (8) was obtained.

Two hundred milligrams of this material was heated on a steam bath for one hour with 20 cc. of 5% hydrochloric acid. The material dissolved, giving a deep yellow-orange solution and then a yellow precipitate. The yellow precipitate was collected by filtering the hot mixture. This yellow precipitate melted at 297° with decomposition. As the filtrate cooled, a fluffy orange material crystallized. This orange material melted at 270° with decomposition.

A positive test for the methylenedioxy group (9) was obtained.

No reaction was obtained with sodium hydrosulfite.

No methoxyl groups were present in this compound when determined by the Viebock and Schwappach method (10).

This compound was methylated, using diazomethane. The methylated derivative gave a positive reduction reaction with magnesium and hydrochloric acid. It also gave a positive Wilson's boric acid test (8), which indicated that this derivative possesses the flavonol or isoflavonol type of structure, and that a hydroxyl group is present in the 5-position which is resistant to methylation and diazomethane. The average methoxyl content of this compound was 19.23%.

Acetylation of the yellow pigment was attempted, using the acetic anhydride and sodium acetate method, and also using the acetic anhydride and pyridine method. In both cases a pale tan amorphous residue was obtained which was not possible to crystallize. This residue melted in the range 115–117°.

Elementary analysis of the yellow pigment indicated the absence of sulfur, nitrogen, and halogen.

Anal.—Calcd. for $C_{15}H_{14}O_7$: C, 53.70; H, 5.22. Found: C, 53.42, 53.12; H, 5.21, 5.24.

SUMMARY

Pharmacological examination has indicated that the above-ground portion of *Lithospermum ruderale* contains a factor, or factors, which suppresses the estrous cycle of female mice. Attempts to isolate this factor by solvent extraction were unsuccessful.

A determination for the presence of alkaloids was negative.

A Stas-Otto determination for glycosides was negative.

Potassium chloride was isolated and identified.

A waxy residue was isolated and separated into two wax fractions. Saponification of Wax A yielded an acid and an alcohol fraction. The data suggest that it is a mixture of C_{24}, C_{26}, and C_{28} acids (11). The alcohol fraction approximated the known data for a C_{30} alcohol. Saponification of Wax B yielded an acid and an alcohol fraction. The data suggest that it is a mixture of C_{24}, C_{26}, and C_{28} acids. The alcohol fraction appears to consist mainly of a C_{32} alcohol.

An orange-colored flavonol, or isoflavonol, with an auxochromic group in the 5-position has been isolated and partially characterized.

A yellow-colored flavonol, or isoflavonol, which has an auxochromic group in the 5-position has been isolated and partially characterized.

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Book Reviews

Handbook of Dangerous Materials. By N. IRVING SAX. Reinhold Publishing Corporation, New York, 1951. viii + 848 pp. 19 x 26 cm. Price $15.

This book is designed mainly for safety engineers, plant superintendents, and others involved in handling and shipping hazardous chemicals and other materials. It should, however, serve as a useful addition to the library of any pharmacist who might be called upon for advice relative to antidotes for poisons and the treatment of injuries resulting from the improper use of rare or unusual chemicals. The book includes more than 5,000 entries of potentially dangerous industrial materials. These substances are alphabetically arranged, cross-referenced, and indexed according to their generic names, trade mark names, and chemical names. In general, information about each material includes information on toxicity, flammability, storage and handling, physical properties, formulas, and shipping regulations. In most instances, detailed information is furnished on symptoms, treatment, and antidotes, first aid suggestions, personnel safety precautions, fire-extinguishing agents, and other important data. The complete text of the Interstate Commerce Commission Shipping Tariff is reproduced, and a section on radiation and radiation hazards is included. The printing and binding are adequate and the typography has been well designed. The book is provided with a complete general index which enables the user of the book to locate the information he needs promptly.

Pharmaceutical Preparations. 3rd ed. By GEORGE R. CROSSEN and KARL J. GOLDNER. Lea & Febiger, Philadelphia, 1952. 265 pp. 16 x 24 cm. Price $4.

This book is primarily a commentary on the pharmaceutical preparations recognized in the U. S. P. XIV and the N. F. IX. A more suitable title for it would be "Official Pharmaceutical Preparations." The book should be very helpful to students of pharmacy because the students should be able to give their undivided attention to the teachers' discussions of the subject matter without taking extensive notes.

The authors' discussions are concise and clear, and they should be useful as review references to practicing pharmacists as well as instructive to students. However, the following statement is given in the discussion of phenolated calamine lotion: "It has been found that if the polyethylene glycol 400 monostearate be increased from 2 to 4 per cent in calamine lotion, there is no separation when liquefied phenol is added." This is not exactly accurate. The information that the Benzedrex Inhaler contains an amine that is closely related to amphetamine might be further clarified.

This is a useful book that will probably have the general acceptance that was accorded the two earlier editions.

Pharmacology and Pharmacotherapeutics for Dentists. 4th ed. By WILLIAM H. O. MCGEHEE and MELVIN W. GREEN. The Blakiston Company, Philadelphia, 1952. xviii + 550 pp. Illustrated. 16 x 23.5 cm. Price $7.50.

The quality of this book is demonstrated by the fact that it has undergone three revisions since the first edition was published in 1936. In the present edition, the authors have continued to limit the scope of the book to a description of scientifically proved information relating to pharmacology and therapeutics of special interest to dentists. Many parts of the text have been revised or rewritten. Changes in terminology and official names for drugs have been brought into conformity with the latest editions of the *U. S. P.*, the *N. F.*, and *Accepted Dental Remedies.* The therapeutic index has been enlarged, the subject index has been greatly improved by the inclusion of many additional references and cross references, and the subject of prescription writing has been emphasized to a greater extent than in previous editions.

In connection with the newer drugs, their dangers as well as their apparent potential usefulness have been discussed in a restrained and conservative manner. In general, however, the design and style of presentation in the fourth edition follows that of the third edition, a review of which was published in THIS JOURNAL, **37,** 128(1948).

Livestock Health Encyclopedia. Compiled and edited by RUDOLPH SEIDEN. Springer Publishing Co., Inc., New York, 1951. x + 614 pp. Illustrated. 16 x 23.5 cm. Price $7.50.

This book is a comprehensive compilation of definitions and descriptions of terms and symptoms associated with livestock and with diseases of large farm animals, as well as being a general veterinary dictionary. It covers the fields of preventive and corrective veterinary medicine, including the physiological changes, parasitic infestations, methods of treatment, actions of drugs, and methods of application and administration.

The author has included many pictures that illustrate pathologic conditions, that show how preventive measures are practiced, and that illustrate regular husbandry techniques such as castration, barn layouts, lamb docking, etc. The material is well cross-indexed according to scientific and popular terms. Phonetic spelling is used as an aid to correct pronunciation. This will be helpful to practical farmers who acquired their knowledge by experience on the farm.

Helpful shortcuts in arithmetical problems are given, but the choice of 37% hydrochloric acid for the example of calculations of % v/v is unfortunate.

This encyclopedia should be especially valuable to practical husbandrymen, veterinarians, and to pharmacists who are located in areas where they are suppliers of veterinary medicines.

International Pharmacopoeia. 1st ed. Vol. I. World Health Organization, Geneva. Columbia University Press, New York, 1951. xviii + 406 pp. 17 x 24.8 cm. Price $5.

Volume I of the *International Pharmacopoeia* was published in November, 1951, in both English and French. A Spanish translation is in the course of preparation. The intent of the *International Pharmacopoeia*, when the project is completed, as all members of the pharmaceutical profession know, is to promote the establishment of uniform international standards for drugs in universal use. The first volume is considered only a beginning toward the accomplishment of that purpose. Volume II is now in preparation and plans for expanding the scope of the World Health Organization (WHO) activities in the direction not only of uniform standards, but uniform laws regulating the import and export of drugs, are now being developed.

The idea of an international pharmacopoeia is not new. Attempts in this direction were started in 1874, and, in 1902, a conference held in Brussels resulted in an international agreement on the unification of formulas for preparations of a limited number of potent drugs. A second conference, in 1925, also held in Brussels, resulted in an increase in the scope of the earlier international agreement. Actual work on the compilation of Volume I of the *Pharmacopoea Internationalis* was begun in 1937 under the auspices of the League of Nations Health Organization. The project, interrupted because of World War II, was resumed in 1947, under the guidance of the Interim Commission of the World Health Organization. This was accomplished by establishing a WHO Expert Committee on the Unification of Pharmacopoeias, which immediately began working to bring the project to completion. The United States was represented on this Committee by Dr. E. Fullerton Cook, and more recently by Dr. Lloyd C. Miller, Director of Revision of the *United States Pharmacopeia*, since May, 1950.

The *International Pharmacopoeia* is quite similar in form and content to the more modern national pharmacopeias of the present day. Volume I includes 199 monographs on drugs, and furnishes specifications comparable to those of the *United States Pharmacopeia*, for the determination of strength, quality, purity, and identity. Volume I contains 43 appendices giving material of the type found in the general tests, processes, and apparatus section of the *United States Pharmacopeia*. Unlike modern pharmacopeias, no specifications are included in Volume I of the *International Pharmacopoeia* for analytical chemicals used in the various tests laid down in the several monographs. Reagents are listed, but the user is directed to use material of reagent purity which is defined as "of sufficient chemical purity for analytical purposes." This type of specification is, of course, quite inadequate and undoubtedly specifications for reagent chemicals will follow in subsequent volumes of the work.

The monographs in the *International Pharmacopoeia* are only for basic drugs, but dosage forms will probably appear in volumes scheduled for later publication. It is the hope of the World Health Organization that the *Pharmacopoea Internationalis* will be useful to countries which do not have a national pharmacopeia and to those whose national pharmacopeias need revision to bring them up to date. It is quite possible that the *International Pharmacopoeia* will fulfill a useful purpose in this respect. It seems rather doubtful that the *International Pharmacopoeia* can accomplish as much as it hopes to in establishing uniform names for drugs in all parts of the world. The titles of the drugs included in Volume I are in Latinized forms which should be intelligible to most pharmacists in any part of the civilized world. On the other hand, as new monographs are developed, greater difficulties will probably be encountered than in the case of the first 200, all of which are more or less well established in most countries. The *Pharmacopoea Internationalis* should be of interest to all pharmacists who are concerned with the dispensing of drugs and pharmaceutical preparations.

Aids to Pharmaceutical Chemistry. By WILFRED A. MUTEHAM. Bailliére, Tindall and Cox, London, 1951. Distributed by Williams and Wilkins Co., Baltimore, Md. 347 pp. 10.3 x 16.3 cm. Price $2.

The title of this little book, which is published in Great Britain, is misleading. The author states that "pharmaceutical chemistry is a specialized branch of chemistry that tends to confuse the student of it for the first time." This might be true if the student did not first acquire the proper fundamental background in organic and inorganic chemistry. The limited scope of the book is indicated by the statement: "As pharmaceutical chemistry is the chemistry of the British Pharmacopoeia, the logical approach to it is to start at the beginning of that book and deal carefully with each monograph in turn."

The text follows the "logical approach," using equations where these are indicated. The book should be helpful to British pharmacists and chemists, and it might be of limited value as a reference volume in American libraries.

Aids to Pharmaceutical Calculations. By MARY E. BOLTON. Bailliére, Tindall and Cox, London, 1951. Distributed by Williams and Wilkins Co., Baltimore, Md. vii + 96 pp. 10.5 x 16.5 cm. Price $2.

This little book presents a limited review of the arithmetic used in pharmacy. It is designed to be useful to students, but it is a handy book to have in the prescription department. Almost every explanation is given by means of an example, but not all the different types of problems are included. Calculations used in dispensing and the preparation of galenicals are given in 21 pages, while 26 pages are devoted to calculations in quantitative analysis, 8 pages are devoted to calculations in microscopy, and 10 pages cover the use of statistical methods.

The book is published in Great Britain and the choice of material is an indication of the different approach to the subject matter in that country. The earlier editions were published under the title "Aids to the Mathematics of Pharmacy." The appended table of atomic weights of the more common elements apparently was carried over from the earlier editions without checking: 29 elements are listed, and the atomic weights of 7 elements are not in agreement with the 1949 International Atomic Weights.

Twenty-five problems with the correct answers are given. However, the American pharmacist should

obtain different answers to problems involving percentage calculations of solids in liquids. The British calculations given in this book are based upon the assumption that 100 grains of water measures 110 minims. The amount of solute then required to prepare 1 fluidounce of a 1% solution is given as 4.375 grains. The American system, based upon approximately 454 grains (at 25°C.) or 456.4 grains (at 4°C.) as the weight of a fluidounce of water, allows the use of 4.5 grains (official) or 4.6 grains of solid to make 1 fluidounce of a 1% solution.

Penicillin Decade: A Report of Sensitizations and Toxicities, 1941–1951. By LAWRENCE WELD SMITH and ANN DOLAN WALKER. Arundel Press, Inc., Washington, D. C., 1951. 122 pp. 14.5 x 22 cm. Price $2.50.

The purpose of this book is to review the principal published data relating to any untoward effects of penicillin therapy during the first ten years of its clinical use. One trend resulting from penicillin therapy that has been observed is that certain types of organisms, particularly the staphylococci, have shown a steadily increasing resistance to penicillin. The same tendency, but to a lesser extent, has been found to exist among other organisms such as the gonococci, streptococci, pneumococci, and *Spirochaetae*. The conclusion reached by the author is that this resistance factor is not too serious a problem at the present time, but that it may become one in the future.

From the survey, it becomes apparent that sensitivity reactions to penicillin are large in both number and type. According to the record, when crystalline penicillin G became available the reaction rate decreased, but then began to rise slowly after patients became sensitized to the antibiotic after a first course of penicillin treatments. All of the different types of sensitizations and toxicities described in this book are documented by references to the original literature in a bibliography of nearly 350 references. In conclusion, the major author, who is a physician and Medical Director of the Commercial Solvents Corporation, issues a word of caution regarding the indiscriminate use of penicillin or any other antibiotic. This caution statement reads as follows: "*Antibiotics are only of value for the treatment of infections due to organisms sensitive to the specific antibiotics being employed.* Not only must the diagnosis be established bacteriologically, but the organisms' sensitivity should be tested in order to determine the most effective antibiotic to employ and the approximate dosage schedule to be followed. Following this procedure, many unnecessary reactions can be avoided. Even more reactions can be prevented if intradermal sensitivity tests are employed, at least in those patients who have had previous antibiotic therapy.

"By desensitization and the considered use of antihistaminics or other detoxifying agents, surely the greater number of reactions recorded in these pages need not be found in the literature of penicillins' second decade. While the actual *toxicity* of penicillin is almost negligible, its ability to sensitize and to cause serious and even fatal accidents should not be minimized. A grave responsibility rests upon the shoulders of the physician for its wise application in clinical practice."

An Outline of the Infectious Diseases of Domestic Animals. By IVAL ARTHUR MERCHANT. Burgess Publishing Company, Minneapolis, Minn., 1951. iv + 356 pp. 22 x 28 cm. Price $5.

This book outlines according to a definite pattern the important diseases of domestic animals, excepting the diseases of poultry. The outlines are composed of concise and clear statements under the following main divisions: Introduction, Etiology, Epizootiology, Symptoms, Pathology, Diagnosis, Prognosis, Treatment, Prophylaxis, State and Federal Regulations, Public Health Relationships, and References for Study.

Dr. Merchant, who is Head of the Department of Veterinary Hygiene, Iowa State College, prepared this book primarily for the use of students of veterinary medicine. Its use eliminates the need for notetaking during class lectures and provides more time for discussion of the subject matter. That this subject matter covers an important item in our agrarian economy is attested by the itemized losses of livestock and poultry caused by diseases, parasites, and pests in 1942 amounting to 418 million dollars.

A chapter is devoted to a comprehensive outline of the organization and functions of state and federal control agencies. In the midst of the federal bureaucratic divisions of animal industry appears the following: "(g) Adequate authority and funds 1906. $3,000,000,000 for meat inspection, etc." This might be misleading to someone who would use this book as a reference, without the accompanying explanatory lectures by the author.

Space is allowed for additional notes on each page. The index which is appended might have been more generally cross-indexed for easier reference.

This book should be valuable to anyone interested in animal husbandry and the practice of veterinary medicine. It can serve as a brief refresher review for the practitioner as well as a basic text for the student. It can be useful to pharmacists who are located in rural areas and to those who carry veterinary supplies and medicines.

BOOKS RECEIVED

International Pharmacopoeia. 1st ed. Vol. I. World Health Organization, Geneva. Columbia University Press, New York, 1951. xviii + 406 pp. 17 x 24.8 cm. Price $5.

Handbook of Dangerous Materials. By N. IRVING SAX. Reinhold Publishing Corporation, New York, 1951. viii + 848 pp. 19 x 26 cm. Price $15.

Livestock Health Encyclopedia. Compiled and edited by RUDOLPH SEIDEN. Springer Publishing Co., Inc., New York, 1951. x + 614 pp. Illustrated. 16 x 23.5 cm. Price $7.50.

An Outline of the Infectious Diseases of Domestic Animals. By IVAL ARTHUR MERCHANT. Burgess Publishing Company, Minneapolis, Minn., 1951. iv + 356 pp. 22 x 28 cm. Price $5.

Textbook of Organic Chemistry. 3rd ed. By GEORGE HOLMES RICHTER. John Wiley & Sons, Inc., New York, 1952. vii + 762 pp. 15.5 x 23.5 cm. Price $6.75.

Pharmaceutical Preparations. 3rd ed. By GEORGE E. CROSSEN and KARL J. GOLDNER. Lea & Febiger, Philadelphia, 1952. 265 pp. 16 x 24 cm. Price $4.

The Essential Oils. Vol. V. By ERNEST GUENTHER. D. Van Nostrand Company, Inc., New York, 1952. xvii + 507 pp. Illustrated. 16 x 23.5 cm. Price $9.75.

Thiophene and Its Derivatives. By HOWARD D. HARTOUGH. Interscience Publishers, Inc., New York, 1952. xvii + 533 pp. 16 x 23.5 cm. Subscription price $15; list price $16.50.

1952 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1952 Chilean Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1952 Award, nominations must be received on or before August 1, 1952.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented annually at the annual meeting of the ASSOCIATION.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. In addition to the sum of the award, the recipient will receive an allowance of not more than $250 to defray his expenses in attending the meeting.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION'S Council. The present committee includes Justin L. Powers, chairman; Louis Gershenfeld, Charles O. Wilson, Harvey B. Haag, Heber W. Youngken, John C. Krantz, and Frank O. Taylor.

The award is now in its fifth year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist; the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, and the third was Dr. C. P. Leblond, Professor of Anatomy, McGill University, Montreal, Canada. The fourth award will be shared jointly by Dr. George Moore of the University of Minnesota Medical School and Dr. Moses Ashkenazy, Chief of Neurosurgery, Veterans Hospital, Houston, Tex., and will be conferred during the 1952 Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

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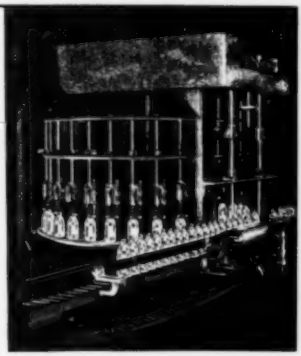

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